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An Introduction to the Theory and Use of the Microscope

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WITH TWENTY-NINE FIGURES IN THE TEXT AND THREE PLATES

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FOREWORD.

THE inspiration of this brochure was the institution of lectures and practical work on Microscopy as part of the class of Medical Physics in the University of Aberdeen. It was felt that a small textbook covering the work of the systematic lectures would be helpful to the student and might aid him to realize the capabilities, limitations and proper method of use of the instrument.

A chapter on the elementary mathematical treatment of certain problems discussed in the text has been added.

It is hoped that the work will prove of value to all students who require a microscope in their studies as well as to those amateur microscopists who wish to understand the fundamental principles on which Microscopy is based.

C. R. M.
H. D. G.

January, 1928.

CONTENTS.

AN INTRODUCTION TO THE THEORY AND USE OF THE MICROSCOPE.

THE function of the microscope is to reveal detail of the structure of objects too small to be visible to the unaided eye. This end is attained by the use of a series of lenses which make the object appear magnified to the observer. The magnification to be of value must extend to the finest detail of the object and each successive stage of magnification should reveal structure invisible without its aid. So-called " resolution " of detail in an object does not of necessity result from mere optical magnification. Magnification is necessary to attain it, but other factors are involved. It is the aim of this brochure to explain the principles of microscopy and the manipulations by which resolution is obtained. For these purposes it is essential to consider first certain properties of the simple convex lens, and some of the conditions governing the visibility of objects.

THE SIMPLE CONVEX LENS.

A convex lens can make all rays falling upon it which diverge from one point converge to another point on the opposite side of it by bending each ray to the proper

extent through refraction at the lens surfaces. In Fig. 1, light rays diverging from O, after passing through the lens L, converge towards I and after crossing at this point diverge again. O is called the " object point " and I the " image point ". Every object point has a

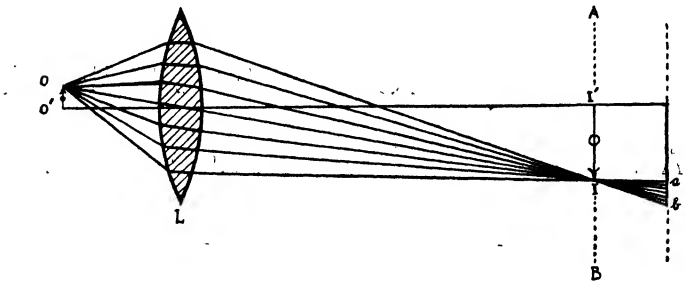


FIG. 1.—Diagram showing the formation of a real image by a convex lens.

corresponding image point—the two are said to be " conjugate "—and the position of the image can be calculated from the laws of Optics. Here two facts only need mention. (i). All rays travelling towards the centre of the lens are undeviated. The image therefore forms on the continuation of the line joining the object point to the centre of the lens. (ii). As the object moves away from the lens the image moves towards the lens. Thus Fig. 14 shows that when an object moves from O ' to O the image moves from I,' to I. When the object has moved to such a distance that it may be regarded as practically at infinity, the image has approached to a definite distance from the lens which is called the " focal length " of the lens (Fig. 2). The point on the axis at a distance from the lens equal to the focal length is termed the " principal focus " and all rays travelling towards the lens parallel to the axis converge to this point after refraction. The focal length is one of the principal optical characteristics of a lens. It depends on the curvature of the surfaces and the refractive index of the material of which the lens is composed—the greater the curvature and the greater the refractive index of the

lens, the shorter is the focal length. The focal length can be calculated when these quantities are known.

The shorter the focal length of a lens, the greater is its converging power and the greater is its ability to magnify. The power of a lens is commonly expressed in " diopters " and is obtained by dividing 100 by the focal length of the lens measured in centimetres. This

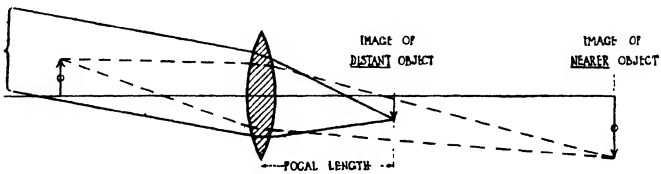


FIG. 2.—Diagram showing the formation of images of objects in different planes. As the object approaches the lens from infinity the image moves away from the lens.

unit is not used in microscopy. The focal length of compound lenses, such as objectives, used with the microscope, is given in millimetres or in inches or fractions of an inch.

Formation of the Image.—Let us suppose that a paper screen is placed in the position A B relative to the lens L (Fig. 1) and that O is a point on the filament of an electric lamp. The point I is the image point of O. It is seen to be formed by a large number of rays proceeding from O being received by the lens and concentrated at I. Hence I is brightly illuminated. Similarly every other point on the filament between O and O′ will form a brightly illuminated image point on the screen. A picture of the filament is thus built up point by point. The image will be sharp and distinct only if the screen is in the position conjugate to the filament. If the screen is moved slightly backwards or forwards from this position, the illuminated patch due to rays from O will be a small disc (ab) and, as other points of the filament will form similar discs, the image will consist of innumerable overlapping discs and will present a blurred appearance.

It is said to be " out of focus." The further the screen is moved from the conjugate position the larger will the discs be and the more indistinct will the image become. Similarly, when an object in a given plane is " in focus " on a screen, objects in front of or behind that plane will be " out of focus " and will appear more or less blurred.

For a perfect image to be possible it is necessary that the lens should bring all rays coming from one point together to a single point. This condition is only approximately fulfilled by a lens consisting of a single piece of glass. The images formed by such a lens show certain imperfections which become more obvious as the image is more critically examined. These departures of the simple lens from the ideal are termed " aberrations ". They have to be " corrected " by special design in lenses used in the microscope. (See p. 11).

The Influence of the Eye.—The eye forms part of the optical system in Microscopy. The greatest refraction of light rays entering the eye occurs at the surface of the cornea and further refraction follows in the crystalline lens situated just behind the iris. As the retina is at a fixed distance from the lens the eye may be compared to a fixed focus camera. It differs, however, from such a camera in that the crystalline lens by the action of the ciliary muscle can alter its curvature and therefore its power. When the muscle is relaxed distant objects are seen clearly. When the muscle is contracted, the curvature of the crystalline lens is increased and objects nearer the eye are brought into focus on the retina. The contraction of the ciliary muscle is limited so that clear vision is not possible for objects closer than a certain limiting distance. The nearest point of clear vision is called the " near point ". It varies somewhat with different individuals and generally recedes with advancing years. Ten inches (250 mm.) is the conventionally accepted distance for optical calculations.

Measurement shows that an average eye can see as

separate two points on an object when the angle formed by two lines joining these points to the eye is about one minute of arc. The limiting angle is formed at 10″ by two lines or points $\frac{1}{200}$″ (0.1 mm.) apart, and an individual with average sight can distinguish two lines or points so separated at this distance. Individuals with very keen vision can resolve lines and points as close as 0.05 mm. The perception of separate points is due to their images falling on different receptive cells (cones) in the retina. Two image points falling on the same receptive cell are not distinguished.

The refractive media of the eye exhibit the " aberrations " inherent in lenses but owing to the structure of the crystalline lens these aberrations are corrected to a high degree. The defects in the retinal image caused by these aberrations are of the same order as the limit of acuity of vision. The purity of the image is not influenced by "shortsightedness" or "longsightedness". The gain or loss of magnification due to them is relatively trifling. Defects of the eye, such as astigmatism, however, which interfere with the purity of the retinal image, must be corrected by suitable spectacles or by special lenses adapted to fit over the eyepiece if the best results are to be obtained.

Visibility of Objects.—For objects to be visible, one of two physical conditions must be satisfied. There must be either—(i) a difference of refractive index between the object and its surroundings; or (ii) a difference in colour.

Difference in refractive index gives what may be termed an " outline picture ". If a piece of transparent colourless glass, say a glass ball, of refractive index 1.52 be placed in a transparent colourless liquid of the same refractive index, it will be invisible. If the liquid be changed for another of different refractive index the glass ball will become obvious and its outline will become more distinct the greater the difference between the refractive

index of glass and liquid. The influence of the refractive index of the medium on the visibility of objects is shown in Plate I., and the way in which an outline picture is formed in Fig. 3. This figure represents a tiny glass bead illuminated by a parallel beam of light acting as an object in the microscope. Rays A and B which just pass the edge of the bead go on undeviated, but ray C

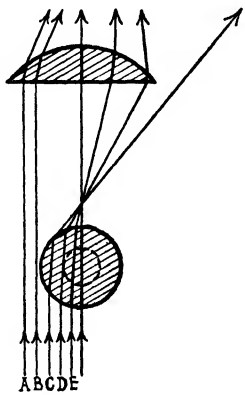


FIG. 3.—Diagram showing the formation of an "outline picture". No ray which enters the object to the left of ray D can reach the lens.

is refracted and does not enter the lens of the microscope nor consequently the eye of the observer. Ray D being deviated less is caught by the lens of the microscope. Thus there is a part of the edge of the bead from which no light enters the lens and this part therefore appears black. Unstained starch grains, air bubbles, etc., are visible in a similar way; and similar principles apply even to minute details of structure.

If the glass bead is coloured, say red, it will be visible even in a medium of the same refractive index merely in virtue of its colour. This will be deepest in hue where the thickness is greatest and in the case of a spherical bead it will gradually fade towards the periphery, which will show no distinct contour. The visibility of a "colour picture" is thus fundamentally different from an "outline picture". As tissues are commonly stained, often differentially stained, with dyes

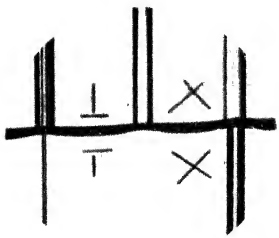
PLATE I.

Effect of Refractive Index of Medium on Image Formation and Visibility.

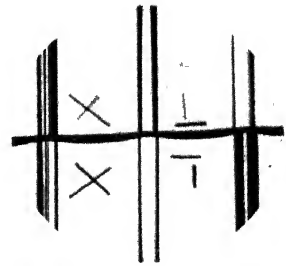
Photographs of (i) thermometer tubing (left), (ii) glass rod (centre) iii) quill glass tubing (right), half immersed in different media containec n a parallel sided glass cell 1 cm. deep. They are typical "outline ictures." Compare the appearance of the objects in the upper half (air nd the lower half (liquid medium).

The horizontal and diagonal lines are strips of black paper on the ront and back of the cell (distance apart 1.3 cm.) Their relative distinct ess—the contrast is lessened by the reproduction—shows the effect o he medium on depth of focus.

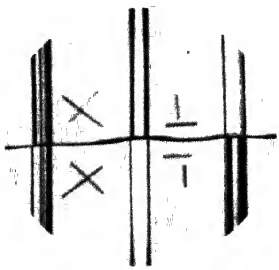
Taken with a lens of approximately .25 N.A., (A), (B) and (C) luminated by parallel light, (D) by convergent light.



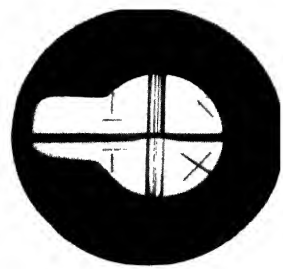
(A) Refractive Index of Medium 1·52. Upper half air. The glass is invisible in the liquid medium and the air in the bore of the right and left tubes alone shows, giving a strong outline picture. The strips of paper are more distinct in the lower half.



(B) Refractive index of medium 1·66. The glass is visible in the liquid medium, but the outline picture is different from that in air, or in medium (A). The depth of focus is the greatest of the series.



(C) Refractive index of Medium 1·33. Appearance in general is similar to (B), but the outline picture is different in detail.



(D) Refractive Index of Medium 1·66 (Convergent Light). The outline picture is sharper than in (B). This figure also illustrates critical lighting. A condenser has been adjusted to form an image of the light source (a "Fullolite" electric bulb) in the plane of the object.

to show their component parts or the structure of individual cells and are usually mounted in a medium of different refractive index from themselves, these objects are visible both as an outline picture and as a colour picture.

The Simple Microscope.—If we could give the eye extra power of accommodation it would be able to see clearly objects closer to it than is normally possible and, since the resulting retinal image would be larger (cf. Fig. 4), it would be able to discern finer detail in

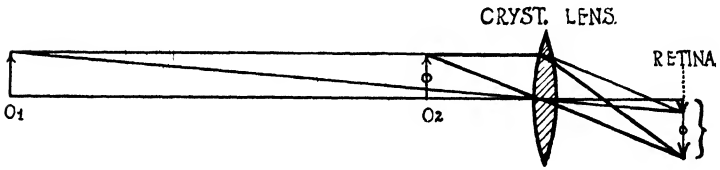


FIG. 4.—Diagram showing the formation of images of near and distant objects on the retina. The size of the retinal image increases as the object approaches the eye, the image being kept sharp by increasing accommodation.

the object. This extra power of accommodation may be obtained by holding a convex lens in front of the eye. A lens so used forms the Simple Microscope. Calculation (see p. 74) shows that with the eye at rest, i.e. adjusted for distant vision, the magnification is $\frac{D}{F_E}$ where F_E is the focal length of the lens used, and D is the nearest distance of distinct vision. With the eye accommodated for 10″ the magnification is slightly greater, being $1 + \frac{D}{F_E}$. A lens of 25 mm. (1″) focus would thus give a magnification of ×10 with the unaccommodated eye and of ×11 with the accommodated eye.

The course of the rays passing through a lens and

entering the eye is shown in Fig. 5. The rays coming from two neighbouring points O and O′ are deviated by the lens and made to enter the eye along paths which they would have followed had they come from two points I and I′ further apart than their actual origins. The retinal image of O O′ thus corresponds to that of the object increased to the length I I′. It follows that details in an object too small to be visible to the unaided

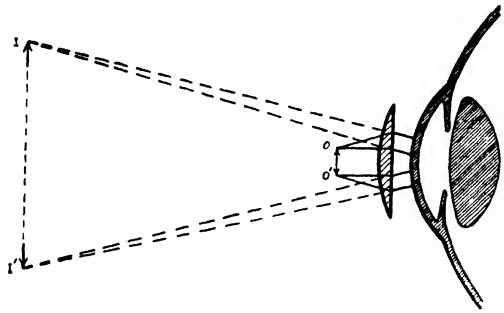


FIG. 5.—Diagram illustrating vision through a simple magnifying glass. The image II′ seen is a virtual image of OO′.

eye may be seen by the help of such a lens. The image I I′ is said to be a " virtual " image—it cannot be projected on a screen like the " real " image formed by a photographic lens. A virtual image is formed by a convex lens when the object is placed closer to the lens than the principal focus.

Simple microscopes are generally used for the observation of objects by reflected light, and the limit of magnification obtainable under such conditions is approximately twenty diameters (× 20). Higher magnification, up to × 50, could be attained for objects which can be illuminated by transmitted light, but further magnification is limited by the great curvature and consequent smallness of the lens necessary to allow an object to be brought sufficiently near its centre to be in focus and by the difficulty of providing adequate

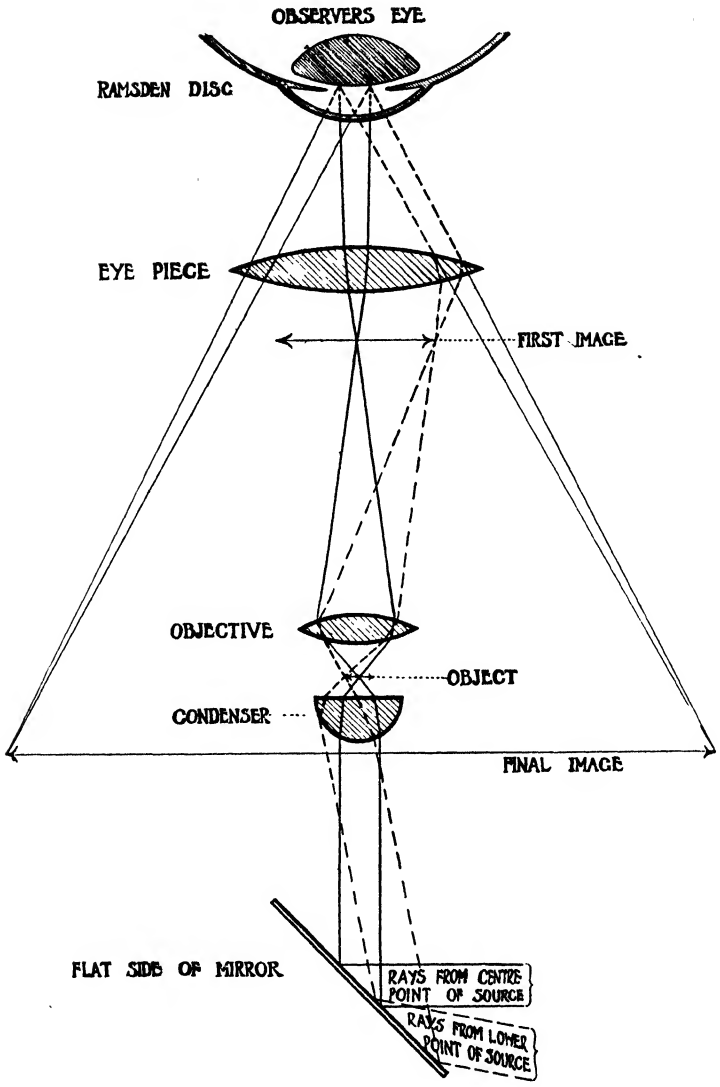


FIG. 6.—Diagram of the course of light rays through a microscope. For simplicity and clearness the proportions of different parts of the instrument have been distorted.

lighting. High magnification therefore necessitates the use of the Compound Microscope.

The Compound Microscope.—In the compound microscope magnification takes place in two stages by two separate lens systems. The first, the " objective ", forms a real magnified image of the object. This is further magnified by a second lens system called the " eyepiece " or " ocular ", which acts on the first image as does a simple microscope on a real object. The two stages of magnification may be followed in Fig. 6. If the first image produced by the objective is magnified twenty times, and the eyepiece magnifies this image a further five times, the total magnification given by the system is clearly × 100. The final magnification is thus the product of the two factors, the objective magnification and the eyepiece magnification.

As efficient illumination of the object is required for its observation, it is necessary with high magnifications to employ a third lens system, called a condenser, to concentrate light on the object (p. 27). The objective eyepiece and condenser must be held in steady and correct alignment and at proper distances apart. These distances and the distances of the objective from the object must be capable of precise adjustment, and the arrangement must be convenient for the observer to manipulate. These requirements are generally fulfilled by the " stand ", which consists of a firm base or foot supporting a limb carrying the body-tube, the stage, substage and mirror. The body-tube has generally an inner sliding " draw-tube " for adjustment of the distance between objective and eyepiece to the proper " tube length ".

THE LENSES OF THE MICROSCOPE.

IT has been shown that image formation depends on the lens bringing together to one point all rays which originally diverged from one point on the object and that owing to " aberrations " a single lens does not do this exactly. In consequence the images produced by it are to some extent blurred and imperfect. These aberrations are of two main types, chromatic and spherical.

Chromatic Aberration.—The refractive index of substances is different for rays of different colours and therefore the deviation produced by a lens of a red ray is different (less) from that of a blue ray which entered the lens along the same path. Since white light is a mixture of rays of all colours, white light emerging from an object point converges not to a single image point but to a series of points spread out into a short linear spectrum along the axis (Fig. 7). This is called the " Primary Spectrum ". As a result of this aberration the image of a white point on a screen is nowhere perfectly sharp, but is edged with coloured fringes. This defect is corrected by replacing the simple lens by a combination of two or more lenses of different materials acting together. Optical glasses differ widely, not only in refractive index, but also in the difference of the refractive indices for red light and for blue. This difference is termed the

"dispersion". Crown glass, flint glass and fluorite, to take important examples, have the following constants:

	Refractive Index n		Dispersion
	Red rays	Blue rays	$n_B - n_R$
Crown glass	1.514	1.523	.009
Flint glass	1.643	1.665	.022
Fluorite	1.432	1.437	.005

If a convex lens of crown glass is conjoined with a concave one of flint glass it is possible to make a combination such that the red and blue rays are brought to the same

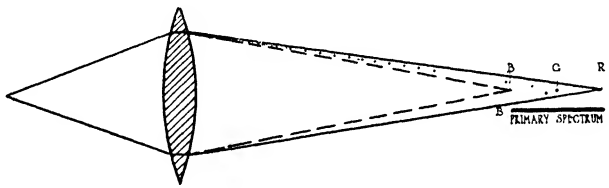


FIG. 7.—Diagram showing the chromatic aberration of a simple lens.

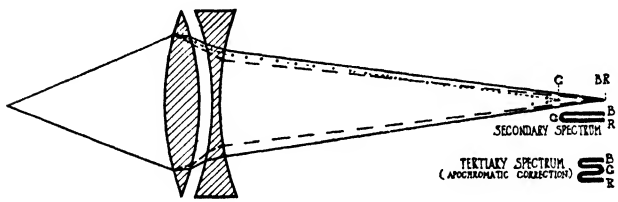


FIG. 8.—Diagram showing the chromatic aberration of an "achromatic" combination, the blue and red rays being brought to the same focus. The effect is a folding over of the spectrum and the formation of a shortened "secondary spectrum" extending from a green to a plum colour. Beneath the diagram showing this folding over is a diagram showing a further folding to bring the red, green and blue rays to the same focus (apochromatic correction). This diagram illustrates the best chromatic correction attained.

image point, as shown in Fig. 8. If we compare this result with the performance of the simple lens the effect may be described as a folding over of the spectrum at

the green. (See diagram Fig. 8.) A shortened and altered spectrum is thus produced which has been termed the " Secondary Spectrum ". This is one step towards contracting it to one point. Correction to this degree gives the " Achromatic Objective " (p. 15).

By a second application of the same principle, using a lens of three components, the secondary spectrum can be again folded over on itself giving still better chromatic correction. This degree of correction (shown diagrammatically below the diagram of the secondary spectrum in Fig. 8) leaves only a slight " Tertiary Spectrum ", and is obtained by introducing the mineral fluorite as a component of the lens system. This mineral is an important constituent of the highly corrected " Apochromatic Objectives ".

Spherical Aberration.—When we examine in detail the paths of the cone of rays entering a lens from an object point on its axis we find that, instead of the ideal represented in Fig. 1., the actuality resembles Fig. 9.

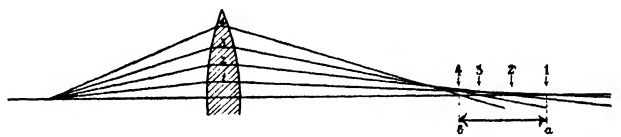


FIG. 9.—Diagram showing the spherical aberration of a simple lens.

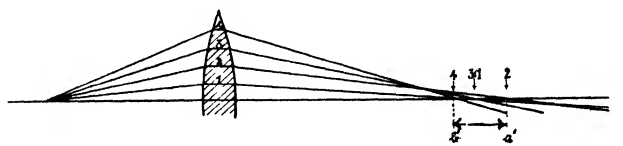


FIG. 10.—Diagram illustrating partial correction of spherical aberration. Rays from zones 1 and 3 are brought to the same focus.

Rays going through the central part of the lens converge to a point further from the lens than the point to which the marginal rays converge. This " spherical aberration " causes a blurred image. By replacing the single lens by a combination of several lenses properly spaced it can be

corrected to a high degree. Fig. 10 shows the central rays and the rays from zone 3 thus brought together on the axis, and in consequence the spherical aberration is reduced from a b (Fig. 9) to a′ b′ (Fig. 10). Such a lens is said to be "spherically corrected for one zone". By a more complicated structure of the lens system rays from a second zone can also be brought to the same point as rays from zones 1 and 3, thus producing a lens corrected for two zones.

The chromatic and spherical corrections are independent; consequently the zonal corrections have to be worked out independently for the various colours. Generally they are made complete with respect to one "preferred" colour, which is often the apple green (wave length 550$\mu\mu$) since this colour is in the part of the spectrum to which the eye is most sensitive.* Zonal correction for the other colours is more or less complete according to the degree of correction it is decided to impart to the lens. Imperfect zonal correction is shown by lack of crispness or by general mistiness in the whole or part of the image.

So far the image formation of an object consisting of one point on the axis of the lens only has been considered. When the object is of finite size so that parts of it are not on the axis a second group of aberrations is involved. Prominent among these is "coma", which causes tails of light to stand out from the side of any brightly illuminated non-axial point. The correction of this defect depends on the fulfilment of a condition known as the "Sine Law" (p. 81) and is obtained by

* The eye is sensitive to only a limited range of radiations, extending approximately from wave lengths 800$\mu\mu$ to 400$\mu\mu$ (μ = 0·001 mm.; $\mu\mu$ = 0·000001 mm). The wave lengths of the spectral colours which constitute white light may be roughly divided thus—Red, 800—600$\mu\mu$; Orange and Yellow, 600—570$\mu\mu$; Green, 570—520$\mu\mu$; Blue, 520—450$\mu\mu$; Violet, 450—400$\mu\mu$. The eye is differently sensitive to these colours. The curve of light perception for small intensities of light rises rapidly through the red, reaches its maximum at about 550$\mu\mu$ in the yellow-green and falls rapidly through the blue.

proper choice and spacing of the lens components. It may be reduced for a preferred colour only or by still more careful design for other colours also. The difficulty of correcting aberrations accounts for the complicated structure of objectives shown diagrammatically in Fig. 15. High power apochromatic objectives may contain as many as nine separate lenses of different materials acting in combination. These lenses have to be worked, polished and spaced with the utmost precision, hence the high price of these objectives. It must be emphasised that the aberrations can never be completely annulled in any lens. The performance of an objective is judged by determining how small the residual aberrations have been made.

OBJECTIVES.

The important characteristics of an objective are—(i) the perfection of its " corrections " ; (ii) its focal length ; (iii) its " numerical aperture ". Other points of interest are the " depth of focus " and " working distance ". The three classes of objectives in use are—(i) the achromatic ; (ii) the semi-apochromatic ; (iii) the apochromatic. They differ as regards the completeness with which the correction of aberration is carried out.

Achromatic objectives are the simplest serviceable objectives. They are computed (a) to give complete spherical correction for one preferred colour (usually apple green) for one zone ; (b) to fulfil the sine condition for the preferred colour; (c) to give freedom from primary colour for one zone. These objectives therefore work best when the object is lit with light of the preferred colour, hence the increase in sharpness of the image when a green glass is inserted between the object and the source of light. When ordinary daylight or other white illuminant is employed these objectives, particularly

the higher powers, give with uncoloured objects images with coloured fringes. Owing to incomplete correction of aberrations the image becomes indistinct (" breaks down ") with high eyepiece magnification. Only the best class of achromatic objectives will bear an eyepiece magnification of × 12. They are however sufficient for ordinary microscopy and owing to their relatively low cost are mainly used.

Semi-apochromatic objectives are a compromise between the highly corrected apochromatic and the cheaper achromatic lenses. In them (a) spherical correction is provided for the preferred colour in two zones with sufficient smallness of the residual " tertiary " aberration in intermediate zones; (b) the sine condition is fulfilled substantially though not precisely for all colours; (c) freedom from primary colour is obtained for two zones. The better correction is partly obtained by employing one, or in the highest powers two fluorite lenses as components of the system and partly by more careful construction. These objectives are therefore more expensive than achromatic objectives. To obtain the best performance from them compensating eyepieces (see p. 25) should be used.

Apochromatic objectives are corrected to the highest attainable degree. Chromatic aberration is corrected to a second stage so that the secondary spectrum left in the achromatic objectives is removed and the barely visible " tertiary spectrum " only remains. The final images formed by the red rays, however, are somewhat larger than those formed by the blue rays and, to compensate for this difference, these objectives must be used with " compensating eyepieces " which correct this slight residual aberration. It is impossible to obtain complete flatness of field with the highest spherical and chromatic correction and with apochromatic objectives it is necessary to employ the fine adjustment to bring the central and peripheral parts of the field successively into focus. (See p. 24).

Focal Length.—The focal length of the objective and the tube length together determine the magnification of the first image. It can be proved that the magnification of the first image is approximately equal to $\frac{\text{tube length}}{\text{focal length}}$ (p. 74) and is therefore greatest when the objective is of short focal length and when the tube length is long. It is not desirable to increase the initial magnification by increasing the tube length, i.e. by extending the draw tube, because objectives are corrected to work with a tube of definite length and with cover glasses of standard thickness. If the standard conditions (see below) are departed from, the quality of the image will be less perfect and the more highly corrected the objective the more noticeable will be the deterioration. The most perfect high power objective improperly adjusted for its best performance may show an image much inferior to that obtained from an achromatic objective of similar power correctly employed.

The tube length for which the objective is computed is often engraved on the mount of the best class of objectives. In modern objectives, when not engraved it may be asumed to be 160 mm.* The standard thickness of cover glass is ·16 to ·18 mm. Variations in thickness of the cover glass from the standard give rise to defects in the image similar to those arising from uncorrected spherical aberration in the objective. These can be corrected, within limits, by alteration of tube length from the standard value. The tube must be shortened for thicker and lengthened for thinner covers than the standard to an extent which depends on the magnification. In the case of oil immersion objectives (p. 20) where there is no refraction at the surface of the cover glass, this adjustment is not required. The draw tube is therefore always set to the value marked on the objective, or to 160 mm.

* See footnote p. 53.

The necessity for alteration of the tube length may be understood from Fig. 11. To minimise spherical aberration, the designer

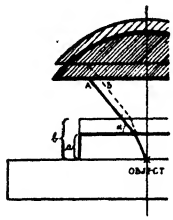


FIG. 11.

in computing a lens has to assume some particular working distance since he has to arrange that a ray entering the lens at a particular angle α will enter by a particular zone A. Let us suppose that the thickness of the cover glass is increased, then the ray at angle α now enters zone B, and to restore the original conditions for this ray, the lens must be moved back. Computation shows that the adjustment required is larger for the peripheral than for the central zones of the lens so that re-focussing the objective alone is insufficient to restore standard conditions of working. Compensation is best obtained when the objective is made to work with slightly lessened tube length. The magnitude of this correction at × 1000 is about 10 mm. decrease of tube length for an increase of .01 mm. of cover thickness.

Although most objectives are corrected for a tube length of 160 mm. (6″) known as the " short " tube, they may on request be corrected for the " long " tube (250 mm. or 10″) or for intermediate lengths. Objectives of the same focal length, as shown by the formula above, give a larger magnification (approximately 50 per cent. larger) on the long tube than on the short tube.

Numerical Aperture.—The numerical aperture of an objective affects (i) its light gathering power and therefore the brightness of the image formed; (ii) the depth of focus and working distance; (iii) the resolving power of the objective. In the case of a dry lens (see p. 20) it is defined by the sine of half the angle of the cone of rays which the lens takes in from an object placed at its principal focus. Thus the N.A. of the lower lens in Fig. 12 is equal to $\sin \alpha = \sin 30^\circ = 0{\cdot}50$. It can be approximately estimated by dividing the radius of the back lens by the focal length of the objective (see

p. 77). Special apertometers are required to measure it accurately.

In Fig. 12 lenses A and B are of the same focal length and therefore give the same magnification when used under similar conditions. But A has a higher N.A.;

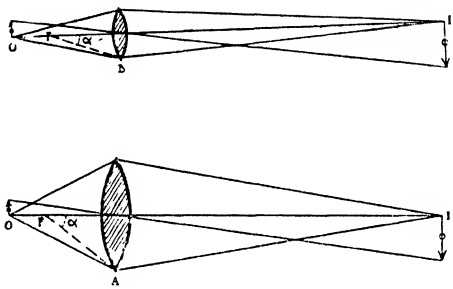


FIG. 12.—Diagram of image formation with a lens of small and a lens of high N.A. The lens of high N.A. gathers a larger number of rays from the object and therefore gives a brighter image. The angle α is a measure of the N.A. F is the principal focus.

it takes in a larger cone of light from the object and therefore gives a brighter image. All the light which forms the final image must come from the part of the object in view. Therefore the light-gathering power of the objective must increase as the total magnification increases if the final image is to be bright enough for visibility. Later (p. 34) it will be shown that increased N.A. is also necessary for the resolution of detail. In the case of objectives of the highest power, numerical aperture adequate to satisfy these two requirements is obtained by adopting the " immersion " method. The space between the front lens and the cover glass is filled with " immersion oil " (or water for a water immersion objective) which is held in place by surface tension. The oil is usually cedarwood oil modified to have as nearly as possible the same refractive index and dispersion as the glass of the front lens and cover slip. The advantage

of this device is illustrated in Fig. 13. The figure shows that when using a dry lens—the only kind so far considered—the surface of the cover glass is really part of the optical system and, as already stated, the designer has to take into consideration the refraction of the rays

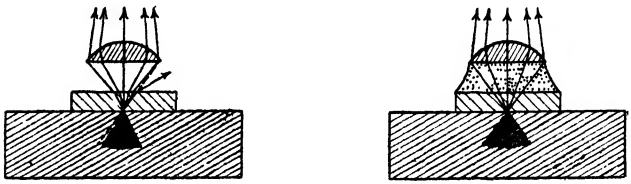


FIG. 13.—Diagram showing the light gathering power of a "dry" and an "oil-immersion" objective. The rays passing to the dry objective are refracted at the surface of the cover glass so that some of the marginal rays fail to enter.

of light when passing from glass into air. In the case of oil immersion objectives, owing to the space between cover glass and lens being filled with a liquid of the same refractive index as glass, the rays are not bent in passing from cover slip to objective. As the figure shows, more light is received by the front lens of the objective, and the immersion objective has therefore a higher effective N.A. The immersion fluid thus increases the effective N.A. Calculation shows that the N.A. of any lens, dry or immersion, may be defined as $n \sin a$, where n is the refractive index of the immersion substance and a is half the angle of the cone of light entering the lens. The refractive index for immersion oil is 1·515, for water 1·33, for air 1·0. Since no angle has sine greater than unity it follows that the N.A. of a lens cannot exceed the refractive index of the immersion substance. Therefore the N.A. of an oil immersion lens cannot exceed 1·5, nor that of a dry lens be greater than 1·0. The full theoretical N.A. is unattainable. In practice the N.A. of

a dry lens rarely exceeds 0·9, of an oil immersion lens 1·40 and of a water immersion objective 1·20.

Note on immersion oils. An immersion oil should not only have the same refractive index as the front lens of the objective but should also have the same dispersive power (p. 12). It is consequently desirable to use the immersion oil prepared by the maker of the objective. Substitutes for a properly adjusted immersion oil produce inferior images even when of the same refractive index since they possess a dispersion different from that of the glass of the front lens.

Depth of Focus.—By depth of focus or penetrating power is meant the thickness of the layers of an object which can be distinctly seen with one setting of the microscope. It is attributable to two causes :—(1) A small amount of diffusion in the final image is permissible if below the limit of visibility, since it is clear that object

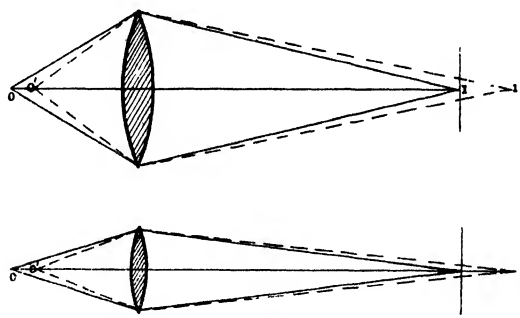


FIG. 14.—Diagram showing that "diffusion depth" of focus is larger with a lens of small than with a lens of high N.A. The unbroken and broken lines show image formation of two axial points O and O' an equal distance apart. The image-disc of O' in the plane of the image of O is seen to be much smaller in the case of the lens of small N.A.

points within a certain axial range of depth will give images in which the lack of perfect sharpness is inappreciable. The " diffusion depth " is smaller, the greater the objective N.A., (Fig. 14) the greater the total magnifica-

tion, and the smaller the refractive index of the mounting medium (see p. 79); (2) Alteration of the accommodation of the observer's eye is optically equivalent to a small change of focus of the instrument and gives rise to " accommodation depth ". This is the most important factor in visual work and practically controls the depth of focus for an observer whose accommodation is normal. Accommodation depth is treated on p. 78 and is shown to be equal to the quotient of the observer's nearest distance of distinct vision and the square of the magnification in use. Thus for a person with a near point at 10″ employing a magnification of 100 the accommodation depth is $\frac{10''}{[100]^2} = \frac{1}{1000}''$. With magnification of × 1000 it has the minute value, $\frac{1}{100,000}''$. As a result of refraction at the surface of the cover slip, a greater depth of focus is obtained with mounting media of high than with those of low refractive index. This point is illustrated in Plate I. where it is shown that strips of paper at a definite axial distance apart give clearer images when seen through a liquid medium than through air, the lens being focussed on a point midway between them. The increased depth of focus obtained by using media of high refractive index is sometimes employed in photomicrography to obtain better images of thick objects.*

Except for such purposes as plankton searching and for stereoscopic effects, depth of focus is generally no advantage. It means a relatively low N.A. and therefore inferior resolution. It is better for most purposes to obtain the fullest resolution in one plane of the object and to focus neighbouring planes by the fine adjustment than to obtain inferior definition through greater thickness.

* The refractive indices of media used as mountants in microscopy are (air 1.0) — water 1.33; glycerine 1.46; liquid paraffin 1.47; oil of turpentine 1.47; cedarwood oil 1.51; canada balsam 1.52; clove oil 1.53; anilin 1.58; styrax 1.63; mono-brom-naphthalene 1.66; piperine 1.68; realgar, approximately 2.4.

Working Distance.—The working distance is the distance between the front lens of the objective and the surface of the cover slip when the object is in focus. The designer endeavours to make the working distance as large as possible, but it is always less than the focal length of the objective, and is smaller the larger the N.A. For high power objectives of large N.A., specially thin cover glasses have to be used. The focussing of such objectives therefore requires the greatest care. The working distance of some typical objectives is shown in Fig. 15.

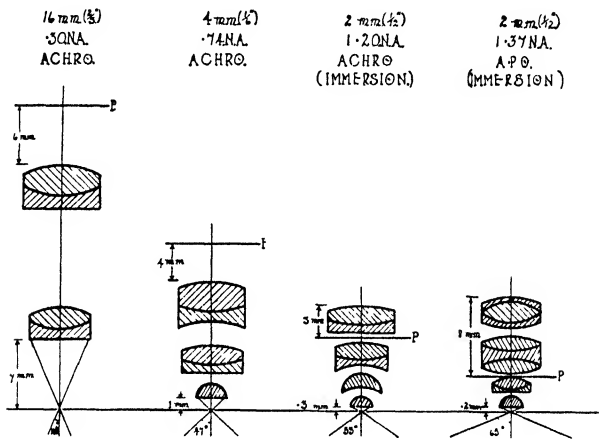


FIG. 15.—Diagram of the optical components of some typical objectives. P is the level of the back focal plane of the individual lenses and its distance from the posterior surface of the back lens is indicated. On the horizontal line representing the object plane the angular aperture of the objective and its working distance are indicated. No cover glass is shown.

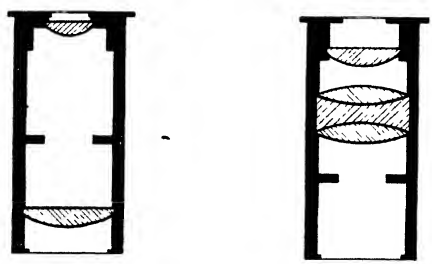
This figure also shows the components of the objectives, the position of the back focal plane, the plane of the object, and the angles of aperture. From these angles, the student will gain some idea of the increased cone of light received by the higher power objectives notwithstanding the small size of the front lens. As explained previously, the light content of the cone is increased in

the case of the oil immersion objectives by the oil contact between cover slip and slide.

Flatness of Field.—Perfect flatness of the final image over the whole visual field is incompatible with the highest degree of correction in objectives. The image is in reality formed at the surface of a sphere the radius of which depends on the nature of the objective. With low power objectives the sphere is large and hence the visual field appears flat, the peripheral and central parts being simultaneously in sharp focus. With well corrected high power objectives the sphere is small and the whole field of view is not in focus at one time. Increased flatness of field can be obtained either by some sacrifice of spherical correction or by the use of eyepieces of special design.

EYEPIECES.

The eyepiece or ocular acts as a simple magnifying glass on the first image formed by the objective. Eyepieces are of two kinds—the Huyghenian or Negative and the Ramsden or Positive (Fig. 16). The Huyghenian



(*a*) (*b*)

FIG. 16.—Diagram showing the structure of (*a*) a Huyghenian or negative and (*b*) a Ramsden or positive eyepiece. The position of the "stop" should be noticed.

eyepiece consists of two lenses fixed at opposite ends of a short tube which slides into the top of the draw tube

of the microscope. The lower and larger is termed the field lens, the upper and smaller the eye lens. Between them, at the focus of the eye lens is a ring of metal or diaphragm called the "stop". The first image is formed in the plane of this stop and its circular edge bounds the field of view. The field lens brings the first image into the plane of the stop. It has an unimportant effect on magnification but aids correction and widens the field of view. The eye lens acts as a simple magnifying glass on this image. In the positive eyepiece, all the component lenses are above the plane of the first image, and the whole system acts directly as a simple magnifying glass on the image below it. The two types of eyepieces can be distinguished by observing the position of the stop which is always in the plane of the first image. In the Huyghenian eyepiece it is between the lenses, in the positive eyepiece it is below the lenses. They can also be distinguished by testing them as simple magnifying glasses ; the positive combination can be used as such, the negative combination cannot be so used except in an inverted position.

Either type of eyepiece may be obtained in the "Compensating" or "Ordinary" series, although ordinary eyepieces are usually made in the Huyghenian form. The two forms can be distinguished by looking through them at the sky or at a brightly lit surface. A compensating eyepiece shows an orange ring and an ordinary eyepiece a blue ring at the extreme edge of the field. Compensating eyepieces are designed for use with apochromatic objectives and are essential to compensate a residual chromatic aberration in the objective (p. 16). They improve the performance of semi-apochromatic objectives and, since they are somewhat under-corrected, to a slight extent that of achromatic objectives of high power.

Ramsden Disc.—When the microscope is in use the light comes from the eye lens of the eyepiece in the form of a bundle of rays which converges and then diverges,

(Fig. 6). If a piece of thin white paper is moved to and from the eyepiece the narrowest part of the bundle will be seen to form a sharply defined bright disc which is an image of the back lens of the objective. This disc is the " Ramsden Disc " and it is at this position that the eye is placed when the microscope is in use. The higher the power of the eyepiece the smaller is the Ramsden disc and the nearer it is to the eye lens (p. 79). Hence with eyepieces of high power the eye must be brought close to the eye lens and must be held very still. Partly on this account high power eyepieces cause greater eye strain than low power eyepieces.

Magnifying Power.—The magnifying power of an eyepiece depends on its focal length as explained in connection with the simple microscope. Powers from $\times 2$ to $\times 40$ may be obtained but for ordinary work powers from $\times 4$ to $\times 12$ are generally employed. Many achromatic objectives will not allow of an eyepiece magnification higher than $\times 8$, owing to defects in the image due to incomplete correction being revealed, and generally, when a higher total magnification is required, it is better to change to a higher power objective.

Ordinary eyepieces are commonly marked with a letter or number instead of with their magnifying power. Usually A or 1 = $\times$ 4 ; B or 2 = $\times$ 6 ; C or 3 = $\times$ 8.

CONDENSERS.

Condensers are employed to illuminate transparent objects and are carried in a substage fitting between the mirror and the stage. For low power objectives of 0·30 N.A. or less efficient lighting can usually be obtained from the concave mirror used without condenser as shown in Fig. 17. For objectives of higher N.A. a condenser is necessary to obtain the best resolution.

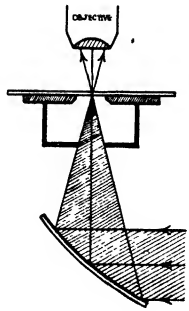
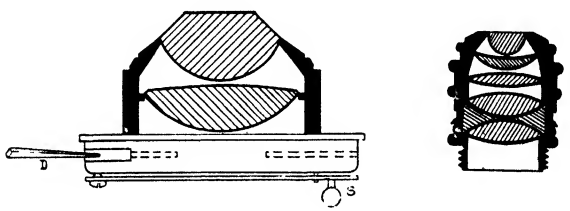


FIG. 17.—Diagram of the course of parallel light rays falling on the concave mirror for illumination of the object. The object is at the principal focus of the mirror.

Condensers are of two types (i) the Abbe or uncorrected; and (ii) the achromatic or corrected (Fig. 18). The Abbe condenser—Abbe termed it the Illuminator—consists of two highly convex simple lenses and, being uncorrected, exhibits marked spherical aberration. This is shown diagrammatically in Fig. 19.



(*a*) (*b*)

FIG. 18.—Diagrams showing the structure of (a) an Abbe and (b) one type of corrected condenser. The lens combinations in different corrected condensers vary. Beneath the Abbe condenser is shown the lever D which controls the iris diaphragm and the ring S to carry colour filters and stops.

The three lens Abbe condenser more recently introduced is better corrected. An achromatic condenser is computed with as much care as an objective and aberrations are reduced to a minimum. Consequently it concentrates all

the light entering it from the source into a solid, so-called aplanatic cone (Fig. 20) at the apex of which is an image of the source of light.

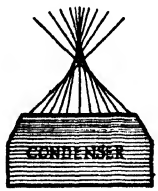


FIG. 19.
Diagram of the bundle of rays from an uncorrected condenser.

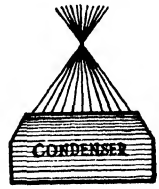


FIG. 20.
Diagram of the "aplanatic cone" from a spherically corrected condenser.

Critical Illumination.—When a perfect image of the source of light is formed in the plane of the object the illumination is said to be " critical ". Critical illumination is necessary to obtain the greatest resolving power of the objective. The appearance of the object plane under critical lighting is shown in one of the figures of Plate I.

The N.A. of the condenser is the N.A. of the aplanatic cone, which as in the case of objectives, is expressed by $n \sin a$. Owing to the aberrations the best two lens Abbe condensers do not give an aplanatic cone when used dry higher than 0·65 N.A.; and many do not give more than 0·5 N.A. A corrected dry condenser of high power has an aplanatic cone of 0·95 N.A. and a corrected oil condenser when in oil contact with the bottom of the slide an aplanatic cone of 1·30 - 1·40 N.A. The cones formed by uncorrected and corrected condensers are illustrated in Plate II.

The cone illuminating the object diverges again and enters the objective. To fill the objective with light and obtain the greatest resolution the condenser used with the objective must have an N.A. at least equal to that

PLATE II.

PHOTOGRAPHS OF THE ILLUMINATING CONES PRODUCED BY VARIOUS CONDENSERS.

The cones of light were made visible by placing the condensers in contact with a block of fluorescent uranium glass. The light source was a Pointolite lamp 10″ from the condenser, the rays being made parallel by means of a bull's eye lens.

Good quality Abbe Condenser. Aplanatic Aperture ·65 N.A.

Corrected Condenser Aplanatic Aperture ·95 N.A.

Corrected Oil Condenser. Aplanatic Aperture 1.30 N.A.

Dark Ground Condenser.

Dark Ground Condenser. Light very slightly excentric.

Cassegrain Dark Ground Condenser. N.A. 1.40

Corrected Condenser with Patch-Stop.

Oblique Illumination.

4 mm, (⅛″) objective of long working distance mounted as condenser.

[*To face p.* 28.

of the objective. It can be shown that the effective N.A. in use is the average of the objective N.A. and the N.A. of the cone of rays coming from the condenser. Thus if an Abbe condenser (N.A. 0·65) is used with an oil immersion objective of N.A. 1·30 the effective N.A. of the system is $\frac{\cdot 65 + 1 \cdot 30}{2} = 0 \cdot 97$. If used with a corrected oil condenser of N.A. 1·30 the effective N.A. of the system is 1·30.

By contracting the iris diaphragm of the condenser, (see Fig. 18), the angle of the illuminating cone of light and therefore its N.A. is diminished. The adjustment of the iris diaphragm to produce a cone to fit the N.A. of the objective is an important manipulation in microscopy and is further discussed on p. 52. On some condenser mounts the N.A. of the cone of light delivered for different positions of the iris lever is engraved.

Focal Length.—The focal length of the condenser controls the size of the reduced image of the source of light formed on the slide. The shorter the focal length the smaller is the image. Generally the image of the light source should be a little larger than the field of view so that the whole field may be illuminated at one time. It follows that a long focus condenser is best adapted for low power work and a short focus condenser, giving greater N.A. and therefore a brighter image and greater potential resolution, for high power work. High power corrected condensers are so constructed that removal of the top lens converts the condenser into an efficient low power condenser. On this account a corrected condenser of approximately 8 mm. focus is most generally useful. It gives sufficient light for the highest powers and on removal of the top lens a sufficient field for low powers. Some condensers of very high N.A. have a very short focal length and consequently require specially thin slides. A dry objective, reversed, forms an efficient condenser if suitably mounted on the substage. It differs

from condensers in being computed to work through a much thinner layer of glass—the cover slip—but this does not seriously affect low power objectives when used as condensers. For critical work with high powers an oil immersion objective may be used as a condenser if the material is mounted for examination between two cover slips.

Dark-ground Condensers.—In dealing with the visibility of objects it was shown that an outline picture is produced when certain light rays do not enter the objective owing to differences in the refractive index of object and its environing medium. There is another method of illumination applicable to objects giving an outline picture in which the object appears luminous on a dark ground. This effect is obtained by using an

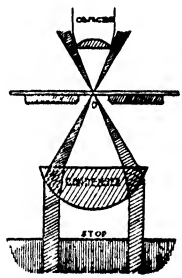


FIG. 21.—Diagram showing the path of rays when a "patch stop," adjusted to give a "dark-ground" effect, is placed beneath a condenser.

illuminating cone of light of much higher N.A. than the N.A. of the objective and stopping out those rays which would normally enter the objective. The course of the rays is shown in Fig. 21. If there is no object at O no light can enter the objective and the field is consequently black, but if an object which can give an outline picture is placed on the stage, some rays, which are passing obliquely through it, will be deviated by its structure, will enter the objective and will form an image of the point at which they were deviated. The outline of

the object and its structure will thus appear as though they were self-luminous, shining brightly on a black background. (Plate III).

To obtain the effect the central shadow must exceed by 10 per cent the N.A. of the objective. For objectives of low N.A. this is usually accomplished by inserting a circular opaque " patch stop " in the swivelled ring below the condenser. (S, Fig. 18). A set of stops is supplied for the purpose or the delicate Travis expanding stop constructed on the iris principle may be used. In lieu of these the top lens of the condenser may be removed and a circular piece of black paper of appropriate size, damped to cause adhesion, may be placed on the upper surface of the lens beneath and the top lens replaced. These methods are only effective for objectives of N.A. less than 0·7. For objectives of higher N.A. special dark-ground condensers are necessary and these must be oiled to the slide. Since the object must be at the apex of the light cone correct thickness of slide and a thin film of material are essential. These special condensers can be obtained to give dark-ground effects with objectives up to 1·40 N.A. All require very accurate centration. Photographs showing the light cone produced by these condensers are reproduced in Plate II.

Besides giving beautiful effects dark-ground illumination is very valuable for the study of living material, especially micro-organisms, because it gives strong contrast and hence visibility without the necessity of staining. It affords a useful training in obtaining proper tube length and is an excellent method for determining the chromatic aberration of objectives. It possesses one disadvantage; it shows dust particles or air bubbles in the slide with confusing brilliance, hence extreme cleanliness in technique is essential.

An adaptation of dark-ground illumination is found in Rheinberg's discs. These consist of coloured gelatine discs with centre and periphery of different colours used as stops. Thus with a central patch of green surrounded

by a ring of red an object may be made to look red on a green background.

Ultra-microscopy. High power dark-ground condensers can be used to show the presence of particles far smaller than can be observed with ordinary microscopy. Thus if a "colloidal solution" (preferably a metal sol) is examined under the microscope with ordinary transmitted light no structure will be seen; but if a dark-ground condenser and high power objective are employed the colloidal solution will appear to teem with brilliant motile points. This appearance is due to the scattering of light by the individual particles and given a sufficiently intense illumination there is no theoretical reason why individual molecules should not be seen by this method. The smallest particle actually demonstrated was a cluster of gold atoms, 6$\mu\mu$ in diameter, in a gold sol. The method gives no evidence of the shape or size of the particles. In this respect it differs from ordinary microscopy which is concerned with the shape and structure of small objects. For exact ultra-microscopy quartz slides and covers are essential since glass cannot be adequately cleansed from surface contamination.

RESOLUTION.

IT is possible to have an enlarged image which, though it may be an exact replica of the gross structure of the object, yet shows none of the fine detail. On the other hand it may be that the detail seen in the image does not exist in the object. These effects arise from a peculiarity in the physical nature of the images formed by lenses. Hitherto the fundamental assumption has been made that the image of a point formed by a perfectly corrected lens is a second point. But when we consider that light is a form of wave motion and the conception of rays only an approximation to the truth, the assumption is found not to be strictly correct. Instead of the image being a mathematical point it can be shown to be a little disc surounded by one or more faint haloes. The diameter of this " diffraction disc " is greater the smaller the N.A. of the lens used and the longer the wave-length of the illuminating light. Assuming a 4 mm. objective of 0·25 N.A. and 1·0 N.A. respectively and green light the disc has for the objective of 0·25 N.A. a diameter about $\frac{1}{2^{5}}$ mm., for the objective of 1·0 N.A. a diameter of $\frac{1}{1000}$ mm. With blue light owing to its shorter wave length the discs are somewhat smaller, with red light slightly larger, while white light gives a composite effect in which all the elementary discs are superposed. This effect is independent of the imperfections of the lens, being a consequence of the nature of light itself. The effects of imperfections in the lens are superposed upon it. The stars of light seen in the ultra-microscope are these discs.

Limit of Microscopic Vision.—Since an object studied may be regarded as composed of a host of minute particles in juxtaposition, resolution of its structure may be considered as involving the separation of two adjacent points. Let us suppose that high magnification with an objective of large N.A. has produced the image of the two adjacent points shown in Fig. 22. The image is two discs. If we decrease the N.A. of the lens the size of the discs will increase, the position of their centres remaining unchanged, and they may eventually overlap, so that ultimately a

FIG. 22.—Diagram illustrating the effect of N.A. on resolution. The disc images I are shown in elevation on the right: at A are shown the larger disc images which would be produced by a lens of small N.A.

series of such points in line will form a bar-shaped image and then the object could not be distinguished from a bar-shaped object. A similar result would occur with an objective of high N.A. if we imagine the objects gradually to approach each other. After a certain critical distance has been passed the disc-images merge and it becomes impossible to distinguish the image of the points from a bar. This critical distance is termed the " limit of resolution " and is equal to the diameter of a disc image divided by the objective magnification. It gives a measure of the finest detail which the lens being used can reveal. It is made small by using a lens of high N.A. and light of short wave-length. For green light and lenses of N.A. 1·40 it has the value ·18 μ. Such resolution can only be attained by correct adjustment and lighting, and then only with simple structures. The resolution in reticulate structures of appreciable depth is smaller than this estimate.

A table of the theoretical number of lines to the inch which can be separated by a given N.A. calculated from the formula $\frac{\lambda}{2 \text{ N.A.}}$ (see p. 82) is published by the Royal Microscopical Society. A few examples given in round numbers are, for white light ($\lambda = 527\ \mu\mu$) – 1·30 N.A. = 125000; 0·85 N.A. = 82000; 0·65 N.A. = 63000; 0·30 N.A. = 29000. For blue rays ($\lambda = 486\mu\mu$) the corresponding numbers are 136000, 89000, 68000 and 31000 respectively.

From this discussion it follows that there is a limit to the useful magnification which a microscope can give. Nothing is gained by magnifying the first image with an eyepiece to such an extent that the disc-images are clearly visible, for then their presence might give rise to total misrepresentation of structure (see Plate III). Theoretical computation (see p. 82) and practical experience show that with ordinary lighting the limit of useful magnification for an average observer is 1000 times the N.A. With objectives and eyepieces of poor quality it is less than this since faulty correction introduces errors larger than those due to disc-images. Magnification within the limit of resolution is " useful magnification " ; beyond the limit it is " empty magnification ", and is so called because it gives no further information regarding the structure of the object.

Ultra-violet Microscopy. Since the limit of resolution depends on the N.A. and the wave-length of light used, attempts have been made to resolve structure further by increasing the N.A. on the one hand and by employing light of short wave-length (violet and ultra-violet light) on the other hand. Increase of N.A. involves the use of media and materials of high refractive indices and has already reached the practical limit. Therefore more recent advances in increasing resolution have been in the direction of using light of short wave-length. Since glass is opaque to ultra-violet rays shorter than $350\mu\mu$ the use of special lenses of quartz or fluorite is necessary throughout the instrument if these rays are to be employed; and since they are not perceived by the retina the image must be photographed. By Ultra-violet Microscopy resolution has been increased

to twice that obtainable with green light. The technique is difficult and the method only useful for certain research purposes.

Abbe's Diffraction Theory. The above treatment has been given because it affords a simple conception of resolution sufficient for its practical application in Microscopy. The theory is not wholly satisfactory and can only be applied in the simplest cases. Further analysis is possible only on the lines of the diffraction theory of Abbe which starts from other premises. Let us suppose that an observer is looking at a distant lamp through the fabric of a wet umbrella. The lamp is seen surrounded by one or more haloes which are due to scattering of light by the reticulate structure of the fabric. A ray of light is scattered by a periodic structure more copiously to right and left at some angles than at others. Apply this fact to an object lying on the stage of the microscope and illuminated by a pencil of light coming up the axis. Most of the light will go straight through but some scattering will take place and if there is regular periodic structure in the object the scattered rays will be concentrated along a series of privileged directions. The finer the structure and the shorter the wave-length of the light used, the greater will be the scale of the scattering and the larger the angles which successive privileged directions make with the axis. The effect is well shown when a suitable diatom (*e.g.* Pleurisigma angulatum) is focussed under the microscope. On removing the eyepiece and looking down the tube towards the objective, the central image of the diatom will be seen accompanied by a set of fainter spectra (called "diffraction" spectra) on each side which are due to the regularly spaced markings of the diatom.

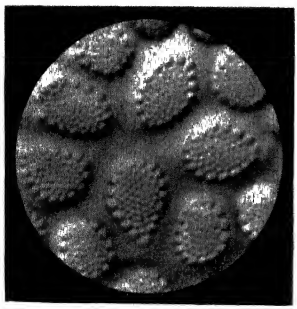
It can be shown theoretically and practically, that if accurate delineation of detail of structure in the image formed by a lens is required, the lens must have a high enough N.A. to gather in from the object the rays going to form these diffracted spectra. The finer the structure the bigger are the cones of rays formed by diffraction and therefore the larger the cone of rays the objective is required to take in. Hence the finer the detail to be studied the higher must be the N.A. of the objective. Computation along these lines leads to similar results to those given by the simpler theory developed in the text. But the diffraction theory goes deeper and is required for the more complete treatment of resolution for which the simpler theory is inadequate. (See p. 83).

PLATE III.

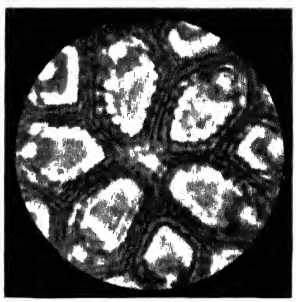
PHOTOMICROGRAPHS SHOWING THE EFFECT OF N.A. ON RESOLUTION.

The photographs are of the central discs of the diatom Coscinodiscus Angulatum. The part photographed is represented by the central black dot, best seen in the top left diatom of the low power dark ground photograph below.

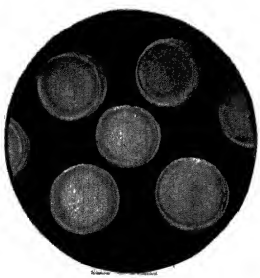
The left figure shows the appearance with N.A. 1·05 and was taken with an oil immersion objective, the condenser N.A. being cut down so long as the purity of the image was maintained. The right figure shows the appearance under ·55 N.A. and was taken with a dry $\frac{1}{5}$" of first class make with a condenser cut down almost to its limit. The figure shows the marked diffraction patterns which produce complete misrepresentation of the real structure, when the magnification is carried beyond the allowable limit. The upper two plates were taken at a magnification of × 800 and photographically enlarged for reproduction.



Oil immersion Objective $\frac{1}{12}$" Corrected condenser. N.A. of System 1·05. Magnification × 2000.



Dry Objective $\frac{1}{5}$". Corrected condenser. N.A. of system ·55. Magnification × 2000.



Low power photomicrograph of group of Coscinodiscus Angulatum under dark ground illumination. Diatoms appear white on black background. Magnification × 25.

[*To face p.* 36.

THE STAND.

THE design of the stand will depend to some extent upon the purpose for which the microscope is to be used. For general purposes a form has been evolved, which has proved convenient, consisting of limb, body, stage, substage and foot.

The Foot.—The foot supports the instrument. It must be of such design as will ensure stability in every position in which the instrument may be used. Two chief types are employed—the horse-shoe and the tripod, with the claw foot as an intermediate form. The horse-shoe form allows of compact design but unless the back projecting toe is carried well out the instrument is liable to be unstable when tilted near the horizontal position. The tripod form is more stable and is usually adopted for the highest class English stands. This form also allows more room for substage apparatus, a valuable feature where a variety of such apparatus is to be used.

The Stage.—The stage is a square or circular accurately planed surface often covered with vulcanite and having a central opening for lighting the object. It should be sufficiently large for all desired purposes. Rectangular mechanical movements and in the case of circular stages rotation is provided in the highest class stands. Attachable mechanical stages can be fitted to a plain stage but they are less satisfactory than the built-in form. Bolting to the stage is the best form of fastening for them.

The Substage.—The substage arrangements control the lighting of the object. They consist of a mirror with plain and concave surfaces suspended in a gymbal or similar fitting on a tail piece near the foot of the instrument and a ring to carry a condenser nearer the stage. The substage ring may consist of a plain tube screwed to the under surface of the stage (" plain underfitting ") or may be attached to a spiral screw fixed to the stage (" screw focussing ") or may work by rack and pinion on a substage fixture. The last form with centering screws in the ring for centering the condenser is usually supplied on the better class English stands and is known as the " compound substage ". It should be rigidly fixed to the limb. Accurate centering of the condenser (i.e. making it co-axial with objective and eyepiece) is essential to obtain the best results from corrected condensers. The Abbe condenser being uncorrected is less sensitive to incorrect centration and may be used with a plain underfitting or with the simple screw focussing ring. The mount of the condenser usually carries an iris diaphragm and a swivelled ring immediately underneath for stops and colour screens (S, Fig. 18).

The Body.—The body consists of a tube carrying the objective and eyepiece. It may work as a sliding fit in a tube attached to the limb but it is usually carried on suitable bearing surfaces on the limb and is moved by rack and pinion actuated by large milled heads. This gives the coarse adjustment. It is an advantage to have the milled heads of the coarse adjustment large and well away from the body. The diameter of the body tube is of importance if low-power photomicrography is contemplated. For this purpose a diameter of 2″ is essential. For ordinary work 1½″ or less is sufficient. The body tube usually carries a " draw tube " (in the best microscopes there are often two draw tubes) into the upper end of which the eyepiece slips. By extending

the draw tube the distance from the objective where it screws into the bottom of the body tube, to the eye-lens —the " tube length "—can be adjusted. The draw tube is generally calibrated in millimetres to enable the setting to be made and in modern microscopes this calibration often includes the depth of the " nose piece ". If this has not been done in the instrument being used an allowance, of say 15 mm., must be made if a nose piece is employed when the tube length is being set.

The Nose Piece.—The revolving nose-piece is a convenient apparatus for rapidly changing objectives. It is screwed to the bottom of the body tube and can be obtained to carry 2, 3, or 4 objectives.

The quadruple nose-piece should be avoided since the weight of four objectives puts a strain on the alignment of the instrument which is undesirable. The chief failing of most nose-pieces for very accurate work is the change in the centration which occurs when the different objectives are swung into position. This defect necessitates centering the condenser with each change of objective. Some nose-pieces are fitted with tangential screw adjustments to correct this failing. In the absence of these, or of a centering substage, the objectives should be so arranged as to give the highest power the best centration. To maintain the centration, the nose-piece should always be revolved in the same direction, namely, clockwise.

More accurate centration can be obtained by using sliding adapters. A "tube slide" screws into the body tube and "objective changers," which are adjustable for centration, slide into it. The adjustment must be made for each objective in its changer. A similar arrangement has been adopted for changing condensers.

The Limb.—The limb is of varied design. It must carry the body, stage and substage in correct and rigid alignment so that the optical parts may be kept accurately centered with one another. It is carried on the foot or on an upright from the foot by an axial joint allowing movement in a vertical plane and it is generally curved in the upper part to afford a convenient handle for moving the instrument. The limb also carries the fine adjustment,

which is usually of one of two kinds, either (i) a lever movement actuated by a medially placed micrometer screw on the upper part of the limb, or (ii) a cam lever or arrangement of cog wheels worked by micrometer screws on the side of the limb. The latter type has usually the finer action and has found favour in recent years but it is often less robust than the simple medial lever which when finely made suffices for every requirement.

The fittings of the microscope are generally made to the standards adopted by the Royal Microscopical Society, e.g. eyepieces (students) 0·9173″ (23·2 mm.) diameter, condenser mounts 1·527″ (38·8 mm.) diameter. Some Continental makers have not adopted the R.M.S. standard for condensers and the condensers of these makers will therefore only fit their own particular stands.

Choice of Stand.—Except for special purposes large and costly stands are a luxury. Work not involving the use of high power immersion objectives can be done with a simple stand provided that it possesses the essentials of rigidity, correct alignment, and accuracy and smoothness of working parts. If an oil immersion objective is required a good rack and pinion coarse adjustment and a fine focussing arrangement of sufficient delicacy are necessary. These adjustments should possess no backlash or lateral movement. Their rigidity can usually be determined by focussing fine structure under high magnification, giving the table carrying the microscope a gentle blow with the hand and noting if any alteration of focus has been induced. An inclinable stand is preferable to one fixed rigidly upright. An inclined stand adjusted to the eye level causes much less fatigue than an upright position when work is prolonged. A working distance of 3″ between stage and body tube is generally sufficient since very low power objectives when required can be screwed into the bottom of the draw tube. For this purpose the draw tube is removed

from the body tube by unscrewing the retaining ring at the top. A compound substage, a "built in" mechanical stage and, if very low power work is contemplated, large mirrors are valuable features of a stand.

A varied choice of models is offered by different makers. If economy is necessary in the first instance it is generally advisable to sacrifice apparatus not immediately required and purchase a stand which will allow further additions to be made when necessary. For ordinary work the compact, so-called Continental model, has many advantages but for exacting work and comfort in working the English form of stand with tripod or claw foot, double draw tubes and compound substage is preferable. The best instrument for the interested amateur is the large English Model. It allows of the use of a greater variety of appartus.

Choice of Objectives.—The number and power of the objectives must depend on the purposes for which the microscope is to be mainly employed. For medical work the commonly used 16 mm. ($\frac{2}{3}''$) and 4 mm. ($\frac{1}{6}''$) objectives, with a 2 mm. ($\frac{1}{12}''$) oil immersion for the study of micro-organisms and the determination of fine structure, provide all that is required. A more generally useful series is a 24 mm. (1″), 8 mm. ($\frac{1}{3}''$) dry and 2.5 mm. ($\frac{1}{10}''$) oil immersion objective. Low power objectives may be needed for observing the gross structure of large objects or for dissection of small objects. They may be obtained as low as 6″ focus but if a low power is only occasionally required the back lens of a 24 mm. (1″) or 16 mm. ($\frac{2}{3}''$) objective may be found sufficient. For its use the front component of the objective must be unscrewed. (On no account must the component lenses of high power objectives be separated as decentration will almost certainly occur). For dissection an erecting prism or lens to produce an erect image and thus

facilitate manipulation is advisable. A suitable binocular microscope e.g. Greenough's (p. 67) is generally employed. The most useful objectives for petrological work are the 24 mm. (1″) and 6 mm (¼″). The same powers together with a 2 mm. oil immersion objective for high magnification are used in metallurgy. The "dry" objectives for this purpose must be specially corrected for observation without a cover slip.

Apochromatic objectives give a more perfect image than achromatic objectives and allow of high eyepiece magnification. Observers used to them do not willingly return to achromatic objectives, but generally speaking, for ordinary work, they are not worth the expense. If the choice is limited to one, preference should be given to a 4 mm. or 8 mm. Semi-apochromatics give almost as good a visual image and are cheaper. They are worth the additional cost over achromatic objectives in the higher powers.

Oil immersion objectives.—The advantages of oil immersion objectives are a higher N.A. and therefore greater resolving power, a fixed tube length with elimination of imperfections due to varying thickness of cover slip, and absence of reflections between front lens and cover glass. The disadvantages are the manipulations necessary in the application of the oil and its removal after use.

The 2 mm. ($\frac{1}{12}$″) is usually the oil immersion objective of choice because it can be given the highest N.A. with the magnification available under ordinary working conditions. The best 2 mm. apochromatic objectives have an N.A. of 1·40. They have, however, a very short working distance and the front lens is very delicately mounted. There is consequently considerable danger of damaging this lens even when cover slips thinner than standard are used. Oil immersion lenses of lower N.A. have a longer working distance and a more robust mounting. The 2 mm. ($\frac{1}{12}$″) of 1·30 N.A. is mainly used, but it, too,

requires very careful focussing and the use of thin cover slips. The 2·5 mm.($\frac{1}{10}$″) which gives the same resolution and has a slightly longer working distance is generally preferable.

The lower power $\frac{1}{4}$″ and $\frac{1}{8}$″ oil immersion objectives have a lower N.A. (0·95) and a much longer working distance. The magnification and resolution given by them are sufficient for most purposes, and in resolving power they are superior to dry objectives of the same nominal N.A. They are convenient to use in conjunction with 2 mm($\frac{1}{12}$″) oil immersion lenses as the change from one objective to the other does not necessitate cleaning the slide.

Oil immersion objectives may be used for dried uncovered objects (e.g. blood films) if object and objective are brought into oil contact. They are not convenient for searching specimens recently mounted in an aqueous medium since during movement of the slide the cover, unless fixed with gummed paper or other adhesive, tends to adhere to the objective instead of the specimen. For such examinations water immersion objectives possess some advantages.

Immersion objectives, since they are specially computed for observation with oil or water cannot be used " dry ".

Choice of Eyepieces.—High power eyepieces except for testing objectives are undesirable. If higher magnification is required it is better to change to a higher power objective which also increases resolution. Low power eyepieces cause less strain and afford more comfort in working. The two most generally useful are a ×4 or ×5 and an ×8 or ×10. If only one eyepiece is acquired ×6 should be chosen. Compensating eyepieces are essential for apochromatic objectives and improve the performance of high power semi-apochromatic and even of achromatic objectives.

Testing objectives. The testing of objectives is a difficult matter. Only an expert microscopist who can arrange the most perfect lighting and can get the tube length on any given object constantly within a 10 mm. range is able to give a trustworthy opinion. A comparison of the performance of different objectives is, however, an interesting and educative exercise and should be attempted by all students who wish to become masters of the instrument. Test objects in common use are the Abbe Test Plate, the proboscis of the blow fly, the podura scale, and a variety of diatoms. Test slides of diatoms giving a graded series which present increasing difficulty in resolution into dots or other fine structure may be obtained. In using diatoms it is well to remember that individual species vary. For experimenting on the resolution of lines one of Grayson's twelve series rulings (5000 to 60,000 lines, or the 10,000 to 120,000 lines to the inch) are valuable. The examination of a bacterium or colloidal particle under dark-ground illumination is also an excellent test for an objective, especially for its chromatic correction.

ILLUMINANTS.

A PERFECT light source which will serve all the purposes of microscopical research is difficult to obtain. The object should be lit with a flat uniform white structureless light of just sufficient intensity to allow the whole of the serviceable N.A. of the objective to be utilised. These requirements appear to be best met by the Hartridge-Williams Axial Illuminator. This apparatus consists of a metal chamber containing an electric lamp which illuminates a plain thin opalescent plate serving as the source of light. The illuminated area can be adjusted by an iris diaphragm and the colour controlled by light filters carried in a swing-out holder. The apparatus is fitted in the place of the mirror. The so-called " tail light " consisting of a small electric bulb with frosted surface and collimating lens to parallelise the light rays and mounted to fit into the substage ring, is also convenient and efficient. Both forms of apparatus require to be run off dry cells or accumulators or through a suitable resistance from the main.

The best extraneous sources of light are the flat wick oil lamp sold for microscopic work and the opal electric bulb. An objection to the opal electric bulb is its spherical surface and, practically, it does not give quite the same degree of resolution as the lamp flame. Light from these sources is usually reflected into the condenser by means of the mirror. A better method, when it can be employed, is to dispense with the mirror and tilt the instrument until the axis of the body tube

and condenser is directed to the light source. The mirror, owing to repeated reflections between the glass and silvered surfaces, tends to impair critical lighting. These reflections are shown by the multiple images of the edge of the lamp flame seen with low magnifications.

The flat wick oil lamp affords on the whole the most economical and satisfactory lighting. For low magnifications a low power condenser or an auxiliary " bull's eye " condenser, to give an enlarged image of the flame, is necessary to provide a sufficiently large illuminated area. The " bull's eye ", which is merely a large powerful plano-convex lens, should be arranged with the flat side towards the light, usually close to the chimney. Its use diminishes the critical nature of the lighting, but for low magnifications this defect is of little importance. Specially corrected lenses to use in place of " bull's eyes " are obtainable to which this objection does not apply. When a band of light across the field is sufficient, the edge of the flame gives the best lighting and is frequently used for critical work. Turned edge-ways the flame is more solid and intense. A common candle affords a fairly efficient source of light if protected from draughts.

An incandescent mantle is less satisfactory as a light source. If used, an opal or ground glass screen should be placed in front of the light to destroy the texture of the mantle in the image. Filament electric lamps in frosted bulbs are still less satisfactory for critical work if the bulb does not obliterate the form of the filament. Daylight is also not satisfactory as it is difficult to control the conditions of illumination. When used the light reflected from large white clouds is best, the condenser being set by focussing some distant structure (telegraph wires or the clouds themselves) on the object and then tilting the mirror to obtain the most satisfactory illumination.

In order to counteract the yellowness of artificial lights a light blue screen is usually interposed between

the light source and the condenser. A deep green or blue-green screen if it can be used gives greater resolution. Monochromatic green lighting may be obtained from a mercury lamp fitted with a special mono-chromat light filter.

For photo-micrographic work a pointolite lamp is usually the best light source. If shortness of exposure is important the more powerful arc lamps may be employed. If length of exposure is not important a flat wick lamp will generally give results equal to those obtained from any other light source.

ILLUMINATION.

Transparent Objects.—When the greatest resolution is required critical lighting is essential. Theoretically the illuminating cone should have as high an N.A. as the objective, but few objectives, except those of the highest quality, will bear a full cone of light owing to insufficiently corrected peripheral zones, and a three-quarter cone is usually best. The brightness of the image must not be such as to cause dazzle. If the light is too intense it is better to reduce it by placing screens of opal, ground or coloured glass between the light and the condenser rather than reduce it by closing the iris diaphragm which diminishes the effective N.A. of the instrument. Circular glasses are supplied to fit into the ring below the iris diaphragm and square glasses to place in holders in front of the light source. (See also Adjustment of Iris, p. 52).

Dark-ground Effects.—For this purpose intensity of light source is all important. Resolution is relatively unimportant since it cannot be obtained to the same degree as with direct lighting. The intensity naturally requires adjustment according to the magnification being used. For observation of living material the

ordinary light sources with, if necessary, an auxiliary condenser usually suffice, but for observing colloidal particles the most intense light available is required. The size of the particles which can be made visible depends solely on the intensity of light that can be used to illuminate them, (p. 32 ; see also p. 17). Exact centration of the light is imperative.

Oblique Illumination.—This form of illumination may be used to show the presence of striae, protuberances and ridges in an object. It is obtained by inserting a stop, having a notch or sector cut out of the periphery, into the stop carrier below the condenser. If a stop carrier is not present the iris diaphragm may be fully opened and the mirror tilted to a sufficient angle. In the Continental mounting of the Abbe condenser the iris diaphragm can be racked out excentrically and rotated to any point. A photograph of oblique lighting is shown in Plate II.

Opaque Objects.—For low magnifications daylight is generally sufficient and owing to its diffuseness gives images without sharp shadows. With artificial lighting the light source is arranged just above the stage and the light concentrated on the object by means of a bull's eye. This method gives sharp shadows with irregular objects. More uniform lighting can be obtained by the use of " silverside reflectors " placed close to the object on the opposite side from the light source, and in some microscopes the mirror can be swung above the stage and the concave side used in a similar way. "Lieberkuhns", which are circular reflectors encircling the objective, can be used if the object is suitably mounted ; but each objective requires a special Lieberkuhn.

For high power work the " vertical illuminator " is employed. This small apparatus screws on to the bottom of the body tube and carries the objective at its lower end. Light is admitted through a lateral opening and

is reflected down through the lenses of the objective on to the object by a cover slip or prism without interference with the image-forming pencils which pass back through it to the eyepiece. A separate light source requires re-adjustment with each alteration of focus of the body tube and therefore the best forms of illuminator carry a small electric bulb with the necessary condensers. This type is largely used in metallurgical microscopy. As critical illumination is just as important for opaque objects as for transparent objects, correct focus and centration of the light, which must fill the back lens of the objective, are essential. The method is only useful for uncovered objects ; with covered objects it is not very successful owing to reflections from the cover slip.

ADJUSTMENT.

To obtain the best results from the microscope co-axial adjustment, proper spacing of optical parts and correct illumination of the object are necessary. The manipulations may be carried out in the following order until facility in rapid adjustment is acquired.

Arrangement of Lighting.

Adjustment of Mirror.—Place the microscope in position with the body tube inclined to the most convenient angle and height for comfort in working. Arrange the light source, if artificial, 8″ from the mirror. If no substage condenser is present remove the objective and eyepiece and look down the body tube from a convenient distance; adjust the mirror so that it reflects an image of the light source centrally and evenly up the tube. The concave side of the mirror is used when no condenser is fitted and brighter illumination is required than is obtained from the flat side. With a condenser the plain side of the mirror should always be used. If a condenser is present and it can be readily swung out of position a preliminary setting of the mirror can be made in a similar way.

Focussing of Condenser.—Put a low power eyepiece and a low power objective on the body tube and a transparent object on the stage; tilt the plain mirror to produce illumination of the object and focus the object.

Focus the condenser so that an image of the light source is clearly seen in the visual field simultaneously with the object (see Plate I). If a bull's eye condenser is being used to enlarge the light source, or if a structureless light source is being employed, a pencil or sharp instrument should be placed in front of the bull's eye or source and focussed. Adjust the tilt of the mirror if necessary.

Centering the Condenser.—This important adjustment can only be made on microscopes possessing a substage fitting with centering screws. On microscopes with a simple ring underfitting or a screw focussing substage the centering—invariably of an Abbe condenser —has been done once for all by the maker. Correct centration is not always attained but if the condenser is distinctly ex-centric it should be rotated in its mount until the best position is obtained. Fitting a centering nose-piece will effect further improvement but this apparatus is less efficient than a centering condenser because it alters the co-axial adjustment with the eyepiece. Exact centration is less important for the Abbe than for the achromatic condenser.

Centration is most easily obtained by removing the eyepiece and looking down the body tube from a convenient distance at the back lens of the objective. Adjust the iris diaphragm so that a distinct rim is seen around the edge of the back lens. If it is not central with the back lens adjust by the centering screws in the substage ring. A more accurate method of centering with corrected condensers is to put a low power eyepiece in position, close the iris diaphragm as far as possible and then rack down the condenser (or rack up body tube) until the iris opening is sharply focussed and centre the opening by the adjusting screws. Condenser and objective must then be refocussed until critical lighting is obtained.

If a halo of light is seen at any sector of the iris opening during this adjustment the mirror must be tilted until the light is uniform around the diaphragm.

Adjustment of Iris Diaphragm.—Remove the eye-piece, look down the body tube from a suitable distance and expand or contract the iris diaphragm until the opening appears about three-quarters the diameter of the back lens of the objective. Few objectives will bear more than a three-quarter cone of light without showing deterioration of the image owing to incomplete correction of their outermost zones.

Fig. 21 explains why the iris should not be too far open. The central unshaded bundle of rays stopped out would produce an " outline picture " of the object which is black on a white field. The outer shaded rays, employed alone for dark-ground illumination, give a white image on a black field. Thus if both act together, as will happen if the iris is open too far, the two factors producing visibility are opposed thus diminishing contrast in the image. The effect is enhanced by the scattering of the excess light and by reflection backwards and forwards between front lens and cover slip. All these effects give rise to a haze of light veiling the image and combine to induce " glare ". The effect may be studied on any object giving a good outline picture. Opening the iris beyond the optimum will impair and may possibly destroy the visibility of the structure.

If the iris is closed too far the outlines may be intensified but resolution will be sacrificed owing to the reduction of the effective N.A. (p. 29) and, in high power work, spurious images may be introduced (Plate III). This intensification is sometimes useful in searching for unstained objects (urinary tube casts, starch grains, etc.) but it must not be employed if fine detail is to be studied. An objectionable practice of students is to rack down the condenser to intensify outline. The same results can be better obtained by closing the iris, a

procedure which does not impair the critical nature of the lighting.

With every change of the objective the iris must be adjusted to meet the altered N.A. of the objective. When no condenser is being used the area of the object lit should be controlled by an iris diaphragm or stops immediately beneath the stage. The optimum condition can be determined by examination of the back lens of the objective after removal of the eyepiece.

Adjustment of Tube Length.

Objectives are usually corrected for a tube length of 160 mm.* and a cover thickness of 0·16 – 0·18 mm. (p. 17). This standard may be assumed for modern objectives unless the data are engraved on the mount. Greater or less cover thickness requires to be compensated for reasons already given (p. 18).

Cover thickness denotes the depth from the surface of the cover slip to the plane of observation. If the object is in contact with the under side of the cover slip and the uppermost plane is being focussed the cover thickness is obviously that of the cover slip itself. If the object is embedded in some mountant at an appreciable distance below the under side of the cover, or if deeper planes of the object are being focussed the effective cover thickness is greater than that of the cover slip.

Some high power objectives are provided with a "correction collar" for compensating aberration introduced by incorrect cover thickness. Rotation of the milled ring on the mount alters the distance of the upper and lower lenses of the objective. Clockwise rotation adjusts for a thicker and counter-clockwise rotation for a thinner cover than normal. With such objectives the

* W. Watson & Sons and Leitz correct their objectives for 170 mm. tube length.

tube length is set at that for which the objective is computed and the adjustment for cover thickness made by rotation of the milled ring. The majority of objectives are not provided with a correction collar and correction for cover thickness must be made by adjustment of the tube length by means of the draw tube. For thickness less than standard the tube length is increased; for greater thickness it is lessened. Correct adjustment by either method is a matter of experience and to obtain this the effect of varying tube length on any good slide showing fine structure should be studied. Within one small range definition will seem crisper and resolution better than at other lengths, and definition will become less sharp as the optimum position is departed from. With experience the way in which fine structure in the object comes into and goes out of focus will be helpful. At correct tube length, for example, an included dirt particle will show a similar appearance as the body tube is raised or lowered by the fine adjustment from correct focus. At incorrect tube lengths the diffusion ring formed by the particle will show a darker centre on one side and a darker periphery on the other side of correct focus.

For the examination of uncovered objects with high power dry lenses specially designed objectives must be employed because extension of the draw tube is insufficient to annul such serious under-correction as is produced by the absence of a cover slip.

With oil immersion objectives correction for cover thickness is not required. The draw tube is consequently set at the length for which the objectives are computed. If the refractive index of the immersion oil differs from that of the cover slip some adjustment of the tube length will be necessary.

Changing Objectives.—In focussing an object the body should be racked down within the working distance of the objective and then with the eye at the eyepiece

racked back until focus is obtained. In the case of high power dry objectives the objective will require to be brought almost into contact with the cover slip and this should be done whilst the objective is observed from the side ; then focus back. This procedure will avoid damage to cover slip or objective. In changing objectives the body tube should be racked back before turning or screwing the second objective into position. Then the fresh objective may be brought within its working distance and focussed as before. Even when objectives are nominally " par-focal ", that is each in focus when swung into position by the nosepiece, it is a wise precaution at first to adopt the procedure given.

In changing to an immersion objective the body tube must be raised and, after placing the objective in position, a small drop of immersion oil (or water, if the objective is a water immersion) is placed on the end of the objective and on the centre of the cover slip and the two drops brought carefully into contact. The object is then focussed by the fine adjustment. If an oil condenser is being used this must be brought into oil contact with the object slide in a similar way before the immersion objective is focussed.

After change of objective it is necessary to adjust the iris diaphragm. It may be necessary to re-centre the condenser.

Changing Eyepieces.—The different eyepieces of one class made by one maker have usually their lower foci in the same plane so that a change from one eyepiece to another does not necessitate re-focussing of the object. If, after the correct tube length has been obtained, re-focussing should be required on changing the eyepiece it is better done by alteration of tube length, which retains the correct working distance already found for the cover thickness in use, than by alteration of fine adjustment (p. 18).

Care of the Microscope.—The value of the microscope as an instrument of precision depends upon its optical parts being clean and its mechanical movements accurate and smooth. The various manipulations must therefore be carried out with care and the glass parts be kept free from dirt. The instrument should be covered when not in use and capped eyepieces, if employed, should be further protected by an inverted pill box or other cover. The instrument should be cleaned immediately after use. Glass parts may be wiped gently with a clean old linen handkerchief—the most easily tarnished parts are the mirror and the upper surfaces of the eye lens and the condenser. Immersion oil should be removed from lens and slide immediately the examination is over, using if necessary a handkerchief damped with xylol. Spirit must not be used as it tends to dissolve the shellac holding the front lens in position. A drop of clock oil should be occasionally placed on the moving parts of the instrument especially the bearings.* If looseness or backlash developes which the user cannot easily remedy the instrument should be returned for overhaul to the makers.

Specks of dirt noticed during examination may be in the eyepiece, objective, condenser, mirror or slide. The situation can be determined by moving these parts separately. The eyepiece may be revolved, the condenser thrown out of focus, the slide moved and the mirror tilted, and the objective may be unscrewed through part of a revolution while looking down the microscope and the position of the offending particle found. The part can then be cleaned with a soft handkerchief, damped if necessary, except when the dirt is on the back lens of the objective when a camel's hair brush should be used. If the dirt is not removable by this means it is desirable to get expert advice.

* For the coarse adjustment of modern microscopes which are only machine finished a thicker lubricant is required.

MICROMETRY.

THE measurement of the size of an object or of the magnification produced by a microscope is most easily made by means of an accurately ruled object slide—the slide micrometer—and a ruled glass disc which is placed on the stop of the eyepiece—the eyepiece micrometer. Slide micrometers may be obtained ruled in parts of a millimetre or in parts of an inch. Each slide micrometer usually contains ten rulings of 0·1 mm. and 0.01 mm. or of 0·01″ and 0·001″ but they can be obtained in other and more extensive series. Grayson rulings are the best. A standard eyepiece micrometer has 50 divisions usually extending over 5 mm. It is placed on the diaphragm of the eyepiece with its thin protecting cover slip at the bottom and it then lies in the plane of the first image. The figures indicating the number of the divisions on the micrometer, when present, should read in their correct position and the divisions should be sharply defined. If not the stop must be pushed slightly up or down in the eyepiece tube until the lines are clearly seen.

To find the size of any structure the stage micrometer is placed in position on the stage and accurately focussed by the objective it is proposed to use. The lines of the stage micrometer are then seen superposed on those of the eyepiece micrometer and the value of the divisions in the eyepiece micrometer can be determined by observing the number of the stage divisions covering a definite number of eyepiece divisions. If exact superposition of

a whole number of divisions of eyepiece and stage micrometres is not observed it may be obtained by alteration of tube length. Suppose 9 divisions of the eyepiece micrometer cover 0·18 mm. the value of each eyepiece division is 0·02 mm. Without alteration of tube length, the stage micrometer is now replaced by the object slide and after bringing into focus by the fine adjustment any structure in the object it is proposed to measure, the number of divisions of the eyepiece micrometer over which this extends is counted and the length calculated. As the value of the eyepiece divisions varies with the eyepiece, objective and tube length in use, it is necessary to determine the value on each occasion. If much measurement with varying magnifications has to be done it is worth while tabulating the value for each combination and tube length to be used. More accurate measurement can be made with the screw micrometer eyepiece, which is a positive eyepiece having a fixed and a moveable spider line in its focal plane. The moveable line is actuated by a lateral milled head, and its distance from the fixed line is read off on a drum calibrated to ·01 mm.

The method gives only linear measurements e.g. the length of the spiral not the length of a spirochete itself. If this complete length is required it is best measured by photo-micrography.

Photo-micrography.—Measurement by the photo-micrographic camera is also linear measurement but by taking a series of photographs the size of structures extending over more than one plane may be determined. If a dark room is available a camera is not actually necessary but it is convenient, and, although an ordinary quarter or half plate stand camera will suffice, a special photo-micrographic apparatus on an optical bench is preferable. The requisites of any arrangement are rigidity, correct alignment of optical parts, exclusion of extraneous light and critical lighting.

In photomicrography the eyepiece is used to form on the photographic plate, a real magnified image of the first image produced by the objective. To obtain the projected real image after the object has been focussed in the microscope by the eye, it is necessary either to draw back the eyepiece slightly from the first image, or to re-focus the objective. For reasons given on p. 18 the former is preferable. "Projection oculars" can be obtained in which this adjustment is facilitated by the provision of a special focussing scale, but they have no other advantage over other eyepieces.

To determine the magnification given by any lens combination the image of the slide micrometer is carefully focussed on the focussing screen and the distance between any two convenient lines accurately measured. If, say, the length of the image of 9 divisions of the 0·01 mm. scale of the slide micrometer is found to be 45 mm., the magnification of the lens combination, with the tube length and camera extension employed, is $45 \div 0{\cdot}09 = 500$ times. If the slide micrometer is now replaced by an object slide and any structure it is wished to measure be accurately focussed by the fine adjustment its length may be determined by measuring it on the focussing screen and dividing by 500. If the length is desired in μ (μ=0·001 mm.) it may be obtained from $\frac{\text{length of image in mm.}}{\text{magnification}} \times 1000$. Thus if the diameter of the image of a red blood corpuscle is 4·0 mm. at a magnification of 500, the diameter of the blood corpuscle is

$$\frac{4{\cdot}0}{500} \times 1000 = 8\mu.$$

If it is desired to determine the magnification produced by an objective alone the image of the stage micrometer with the focussing screen set exactly 10″ from the shoulder of the objective is measured and the length observed between any two lines divided by the known distance of the rulings. If 3 divisions of the $\frac{1}{100}$″ rulings measure 1·5″ the magnification is $1{\cdot}5 \div \frac{3}{100} = 50$. This magnification is given by a $\frac{1}{2}$″ objective at the conventional distance of 10″. If the magni-

fication is insufficient for accurate measurement with the focussing screen at 10″ distance the camera should be extended as far as possible and, from the magnification obtained, that at 10″ distance calculated. If 5 divisions of the $\frac{1}{100}$″ lines measure 1·5″ at 30″ extension of the camera the magnification of the objective at 10″ is $1{\cdot}5 \div \frac{5}{100} \times \frac{10}{30} = 10$. This magnification is obtained with a 1″ objective at 10″ or a $\frac{2}{3}$″ at 6″.

When the objective magnification is known the eyepiece magnification may be approximately determined in a similar way, the focussing screen being however, placed exactly 10″ from the Ramsden disc (the situation of the Ramsden disc can be obtained by moving a piece of translucent paper or ground glass just above the eyepiece). Thus if the magnification of eyepiece and objective together is 80 and the objective gives a magnification of 10, the magnification of the eyepiece is $\frac{80}{10} = \times 8$.

In taking photo-micrographs it is essential to see that no reflections occur in the body tubes of the microscope, especially if no eyepiece is used. The part of the tube carrying the eyepiece is rarely blackened and reflections from this part are a common cause of tails of light, fog and want of contrast. This difficulty may be avoided by lining the tube temporarily with black velvet. The light source must be central and the lighting critical, and the iris diaphragm should be just sufficiently contracted to fill the objective with a three quarter cone of light. After focussing the object visually in the ordinary way, adjust the camera to the microscope, and focus the object on the screen. Protect from all extraneous light and after making the exposure examine the image on the screen to make sure that no movement has occurred.

For general photo-micrographic work apochromatic objectives and compensating eyepieces are best. Good photo-micrographs of colourless objects or objects in monochrome can be taken with achromatic objectives of good quality if monochromatic light is used or a bluish green screen is placed in front of the light source. A green screen improves the definition even of apochromatic objectives. For differentially stained objects a suitable colour screen must be employed and must be chosen

according to whether it is desired to bring out contrast or detail in the object.*

Drawing Eyepieces.—In the absence of a camera or other photo-micrographic apparatus the size of a curved or irregular object can be determined by drawing with the aid of some form of drawing appartus. The apparatus is of varied design but all act on the principle of the camera lucida. The simplest is Beale's Neutral Tint Reflector, which consists of a piece of neutral tinted plate glass set at an angle of 45° when fitted over the eyepiece. The microscope must be placed in a horizontal position and the image is viewed through the reflector and drawn on a piece of paper 10″ from the centre of the reflector. The image is a mirror image. This reversal of sides is immaterial for purposes of measurement but it does not permit of the proper reproduction of some subjects.

Other forms of drawing apparatus consist of a prism or arrangement of prisms fitting over the eyepiece and usually permanently fixed to an eyepiece thus forming a drawing eyepiece. Different forms allow the microscope to be used in an upright position or more usually tilted to an angle of 45°. Abbe's drawing apparatus may be employed in any position of the microscope provided the plane of the drawing paper is at right angles to the optical axis of the microscope. In this form the image reflected from a face of the prism is again reflected on to the paper by a large adjustable mirror. It is important to adjust the apparatus so that the Ramsden disc occupies the plane and fills the aperture between the prisms. A tilting drawing board is a desirable adjunct. Although an easy form to work with it has

* The sensitivity curve of an ordinary photographic plate is different from that of the eye (p. 14, footnote). Its maximum intensity is at 450$\mu\mu$ and it is almost blind to light rays longer than 520$\mu\mu$. On the other hand it is very sensitive to the invisible rays beyond the violet—to about 240$\mu\mu$. It is therefore necessary to use panchromatic plates which are sensitive to all spectral colours, with appropriate screens, when photographing coloured objects.

the disadvantage that it is impossible to include the whole visual field with one setting of the mirror and its re-adjustment to include a part of an object in the peripheral field may alter the scale of measurement. The accuracy of a drawing apparatus and its proper setting may be determined by drawing the image of an object glass ruled in squares. The magnification under the conditions in use must be obtained by drawing the scale of the image projected from a stage micrometer.

The chief difficulty in the use of drawing apparatus is the arrangement of the intensity of the light illuminating the object on the one hand and the image and drawing pencil on the other. Tinted glasses are supplied with the Abbe apparatus to help in this adjustment. As the question of relative lighting is subject to individual variation no general rules can be laid down. The best conditions for any apparatus and arrangement must be determined by trial.

Measurement of Thickness.—The thickness of an object is difficult to determine accurately. It can be approximately measured in an object giving a well marked outline picture by means of the fine adjustment if this is graduated or if the distance of travel of one revolution of the screw is known. It is advantageous if possible to make a scratch mark on the top of the object glass and a similar mark on the under side of the cover slip. These can be accurately focussed and the distance the body tube has had to travel to bring the marks into successive focus acts as a guide. Since the apparent depth of an object immersed in a refracting medium is less than the real depth as a result of refraction at the surface of the medium, the apparent thickness of the object as obtained by this method with a dry lens must be multiplied by the refractive index of the mounting medium to obtain the true thickness. When an objective using homogeneous immersion is employed, this correction is not required.

SPECTROSCOPY.

By means of a spectroscopic eyepiece the microscope can be utilised for observing the absorption spectra of very small quantities of fluids or even of solids. The spectroscopic eyepiece differs in appearance from an ordinary eyepiece but it may be regarded as an eyepiece with an achromatic field lens having, instead of a stop, an adjustable slit at its focus and being surmounted by a train of cemented prisms such as are used in a straight vision spectroscope. It has often a comparison prism covering one half of the slit and is frequently supplied with an arbitrary scale for noting the position of absorption bands.

Fluids are examined in glass cells, a deep form consisting of half an inch of medium barometer tubing ground at the ends and cemented to a slide. Wedge-shaped and other cells are employed. The fluid of appropriate concentration in a suitable cell having been placed on the stage, is focussed with a low power objective and eyepiece. The lighting is then carefully adjusted so that no light which has not passed through the fluid is allowed to enter the objective. Extraneous light passing up the walls of the glass cell or reflected from objective or cell spoils the spectrum and may be avoided by placing a piece of black paper with a punched hole the size of the diameter of the cell on the bottom of the slide. Replace the ocular by the spectroscopic eyepiece; focus the prism until the absorption bands are seen at their best; close or open the slit until they are best defined; adjust the intensity of the light to give the best result.

If for comparison the spectrum of another fluid is desired this fluid is placed in a test-tube or other receptacle which can be accommodated in the fitting at the side of the eyepiece. Pass light through the solution either from a direct source or by reflection from the light source of the microscope by means of the mirror attached to the eyepiece. Adjust the lighting and the slit immediately behind the comparison fluid until the two spectra are of approximately equal brightness. The spectra should be in juxtaposition. If not the comparison prism over the central slit must be adjusted. The scale reading if present is attached to the upper part of the eyepiece in a small horizontal tube. It is reflected from the upper surface of the spectroscope prisms and requires separate illumination.

SPECIAL MICROSCOPES.

THE three chief variants from the ordinary instrument are the metallurgical and petrological microscopes and a form known as the binocular microscope which has been designed to produce stereoscopic effects.

THE METALLURGICAL MICROSCOPE.

The metallurgical microscope is used for the examination of uncovered opaque specimens such as sections of polished metal. It therefore requires no condenser or mirror since the light for illumination must be reflected from above. For high magnifications the light is reflected through the objective, and this is accomplished by means of a vertical illuminator (p. 48) attached above the objective. As the light source of the illuminator, unless self-contained, would be decentered by any movement of the body tube the stage is also made to focus. The objectives must be corrected for use without a cover slip.

THE PETROLOGICAL MICROSCOPE.

The distinctive feature of the petrological microscope is the combination of two separate instruments—a microscope and a polariscope. For convenience in making angular measurements on crystals, the stage, in most models, is made to rotate and is graduated round the margin into 360°, while the eyepiece is fitted with cross wires as lines of reference. The polariscope which is used for producing "polarised light" consists of two Nicol's prisms.* One of these

* A Nicol's prism is a rhomb-shaped crystal of pure transparent Calcite (Iceland Spar) cut diagonally and mounted in such a way that light rays traversing the prism are "plane-polarised" i.e., are caused to vibrate in one plane only.

called the "polariser" is carried in a swing out substage; the other prism the "analyser" being fitted in the tube above the objective (or sometimes, as in the analyser eyepiece, above the eyepiece proper). The polariser and analyser can be readily thrown out of the path of light so that an immediate change can be made from polarised light to ordinary light and vice versa. This arrangement is very convenient in practice because so many characteristic features of minerals are related to their behaviour in polarised light and a ready method of producing it is essential in petrological work. Many minerals and rocks show beautiful double-refraction colours under polarised light and are attractive microscopic objects.

Most petrological microscopes carry also a special system of converging lenses for producing so-called "convergent polarised light". This light is used for the observation of the beautiful "interference figures" yielded by minerals and for other optical tests. Other accessory apparatus such as quartz wedges, quarter wave plates, etc., add greatly to the efficiency of the instrument.

Many of the fittings of a petrological microscope can be adapted to an ordinary microscope. A polariser to fit into the substage ring and an analyser to screw into the body tube and carry the objective can be obtained. The addition of a selenite plate, which is placed between the polariser and the object, produces with some crystals gorgeous colour effects and these are even enhanced by introducing a quarter wave plate,* which produces so-called "circular polarised light."

* A quarter wave or quarter undulation plate is a film of mica the thickness of which is adjusted to retard one of the rectangular components of the incident plane-polarised vibrations a quarter of a wave length relative to the other.

The Binocular Microscope.

In the binocular microscope an attempt is made to obtain stereoscopic vision for microscopic objects. Within limits, stereoscopic vision of microscope objects may be obtained (i) by viewing the object with two separate microscopes held rigidly together at the correct angle of convergence ; or (ii) by dividing, geometrically or optically, in some part of its course, the pencil of rays carrying the image from one objective and diverting the divided rays into separate body tubes adjusted to the proper interpupillary distance. Each of these methods is in use.

Greenough's Binocular Microscope.—This form of binocular microscope has two separate body tubes fitted with paired objectives rigidly fixed at the proper angle. An erecting system is contained in each tube and objects are consequently seen in their natural position. The construction does not permit of the use of objectives of greater focal length than 24 mm. (1″) since higher power objectives, owing to the breadth of the mount, cannot be brought sufficiently close together. Greenough's binocular gives the best stereoscopic effects for the magnifications which can be employed.

Wenham's Binocular Microscope. — Wenham's binocular has two body tubes, one vertical and one inclined at an angle, joined at the bottom and carrying one objective. Below the junction of the body tubes is a special deflecting prism—" Wenham's prism "—covering half the diameter of the tube and capable of being moved into and out of position. This prism divides geometrically, more or less completely, the pencil of rays coming through the objective and diverts the reflected rays into the inclined tube serving the left eye. The half pencil of rays passing up the straight tube to the right eye escape the prism. Owing to reflection from the prism

and to other causes the image is less bright in the left than in the right eye and since the rays passing to the left eye have a slightly longer course than those passing up the straight tube the image on the left side is slightly larger than that on the right. The eyepieces in consequence should be specially paired. To obtain the minimum interpupillary distance the body tubes must be 8″ long. Greater interpupillary distance is obtained by increasing the length of the tubes by a rack and pinion extension of the draw tube. It is consequently advisable to use objectives corrected for the 10″ tube. The illumination requires very careful adjustment to get the best lighting in both tubes.

The Wenham gives good stereoscopic effects with magnifications up to × 150. With increasing magnification the perception of depth and solidity rapidly falls off until at magnifications of × 500 it is scarcely perceptible.

Modern Compound Prism Binocular Microscopes.—These forms divide the pencil of rays coming from the single objective optically, usually by means of a semi-silvered prism contained in a box just above the objective. The short body tubes may converge but in some forms they are parallel or almost parallel and in these the correct interpupillary distance is obtained by a sliding movement actuated by a screw. One of the body tubes usually has a screw-focussing adjustment to compensate for differences in vision of the two eyes.

The advantages claimed for this model are—equal illumination of the image in the two tubes; the use of any power objective; resolution equal to that of the monocular instrument. The last point will be considered later.

Abbe's Stereoscopic Eyepiece.—This apparatus fits into a monocular instrument in place of an ordinary eyepiece. The image pencil is divided in the upper focal plane and special eyepieces—a Huyghenian and a

Ramsden—are required for the right and left tubes respectively. The illumination is unequal in the two eyes.

Binocular Vision.—Stereoscopic vision with the binocular microscope is fundamentally similar to normal stereoscopic vision but is modified in certain particulars by the limitations of the instrument, in particular by the limited depth of the visual field incidental to the magnification and by the limited convergence possible in the visual axes. Normal stereoscopic vision is partly due to sensations associated with convergence of the eyes but more to the formation of slightly different images on the two retinae owing to the object being regarded from two points of view. The part which each of these factors plays in stereoscopic vision with the binocular microscope varies in importance with the type employed. With Greenough's form stereoscopic vision is due mainly to the formation of slightly different images in the two eyes. In Wenham's binocular the two factors vary with the magnification used. With low powers, where the edge of the Wenham prism is approximately in the back focal plane of the objective, the image pencil is fairly evenly divided geometrically and as each half passes to the separate tubes slightly different images of the object are received by the two retinae. With increasingly higher powers the recession of the focal plane (see Fig. 15) from the Wenham prism causes increasingly uneven division of the image pencil, with corresponding diminution in stereoscopic effect due to dissimilar images, until with 4 mm. ($\frac{1}{6}$″) objectives the effect is negligible. With these powers the slight perception in depth obtained with certain objects, e.g. diatoms, is mainly due to sensations associated with convergence and to the phenomena mentioned below.

Accessory factors which are common to all forms of binocular microscope are (i) varying interpupillary

distance of the eyepieces and (ii) association depth due to the formation of shadows by structure in planes more distant than the focussed plane. The latter occurs in monocular vision and is one of the methods by which an artist suggests distance in a picture. This effect is therefore mainly psychical. It seems to be more marked with the Wenham than with other forms of binocular microscope partly owing to the unequal illumination of the two images but mainly because the inner parts of the visual fields are less illuminated than the outer.

Varying interpupillary distance is of greater importance. Equally good stereoscopic vision is obtained if, instead of dividing the image pencil of rays at the focal plane of the objective, the rays are bisected at the two Ramsden circles and the outer half only of these image rays is allowed to pass into the corresponding eye. Similarly, if the interocular distance of the binocular microscope is less than the interpupillary distance, more of the outer rays of the Ramsden disc will tend to pass through the pupil and will intensify the stereoscopic effect. On the other hand, if the interocular distance is greater than the interpupillary distance, more of the inner rays of the Ramsden disc will tend to pass through the pupil and a " pseudoscopic effect," which is a reversal of depth perception, may be produced. Thus a bowl-shaped structure may appear concave instead of convex or vice versa. This appearance, particularly with moderately high magnifications is sometimes very deceptive.

Depth of focus in an objective is of importance in binocular vision with the microscope in that it affects the extent of the planes over which structure can be observed. The optimum depth varies with the thickness of the object, hence to obtain the best results the objective must be adapted to the thickness of the object it is desired to observe. The stereoscopic appearance of a bowl-shaped diatom seen with a 6 mm. ($\frac{1}{4}''$) objective is not observed with a 50 mm. ($2''$) objective, nor is the

stereoscopic effect obtained with objects suitable for a 50 mm. (2″) objective seen with a 6 mm. ($\frac{1}{4}$″) objective. The possibilities of stereoscopic vision are practically reached with a 4 mm. ($\frac{1}{6}$″) objective. The depth of focus of such an objective is approximately 0.002 mm. With powers higher than the 4 mm. the depth of focus becomes of the same order as the limit of microscopic vision for objects in a transverse plane. That of a 2 mm. ($\frac{1}{12}$″) objective is 0·00025 mm. (p. 79). There is therefore no chance for separate images to be formed of a series of planes one behind the other within the depth of focus and perception of depth becomes impossible.

The modern binocular microscope, which divides the image pencil optically, exhibits less stereoscopic effect than the Wenham binocular microscope. In these instruments the image presented to each eye appears to be the same and the stereoscopic appearances observed must therefore be due to unconscious partial bisection of the Ramsden disc, to association depth and other psychical factors, possibly aided in some cases by convergence depth. The chief advantage of these microscopes for general work is the comfort afforded by vision with both eyes. The disadvantage which is common to all binocular microscopes, is their inadaptibility for variations in cover thickness and therefore for obtaining the best resolution under different conditions. The tube length for any one individual is fixed by the design of the instrument or is controlled by the interpupillary distance. Since the interpupillary distance is constant for a particular individual only one tube length is possible with each objective. This disadvantage limits critical work at high magnifications.

Binocular Rivalry.—Some individuals are unable to superpose the two images in the two eyes, especially with the Wenham microscope. There appears to be difficulty in convergence and the images occupy different lateral

positions, with one tilted at a slight angle to the other owing to the absence of the associated reflex adjustment of the oblique muscles of the eye. Continued observation commonly causes fusion of the images but this may not occur. The effect is due to binocular rivalry and can generally be overcome by changing the object or the field and increasing the intensity of the lighting in order to concentrate interest on one point or position. Once proper convergence has been obtained it is maintained for any object as long as the observation continues. If, however, the eyes are taken away from the instrument and focussed elsewhere, the convergence may again become insufficient, and it has to be obtained by a repetition of the process to produce proper convergence.

PHYSICAL PROOFS.

In this chapter it is proposed to give a simple mathematical statement of some of the problems discussed in the text. A knowledge of the elementary properties of refracting surfaces and of thin lenses will be assumed.

Notation.

Objective. Focal Length F_0; Numerical Aperture A; Magnification M_0.

Eyepiece. Focal Length F_E; Magnification M_E.

Tube Length L; Total Magnification M.

Nearest Distance of Distinct Vision $= D = 10''$ (250 m.m.)

(1) Magnification.

(a). **Simple Microscope or Eyepiece.** From Fig. 4 it is seen that the size of the retinal image varies inversely as the distance from the object. A magnifying glass gives the effect of an abnormally close "near point" since it enables an object to be distinctly seen when brought closer to the eye than the normal near point. The conventional near point for optical calculation is 10 inches.

Let the conventional nearest distance of distinct vision be D.

,, new, abnormally close ,, ,, ,, ,, D'.

Then, magnification due to lens $= \frac{\text{size of retinal image with lens}}{\text{max. size of retinal image without lens}} = \frac{D}{D'}$

To evaluate the magnification it is necessary to find D'. Two cases, which also have a bearing on the depth of focus (pp. 22, 78), require consideration.

(i). The observer keeps his eye fully accommodated. The rays entering the lens must then diverge from a point at a distance D if they are to be focussed on the retina. Hence the lens must make the rays diverging from D' appear to diverge from D after passing through it.

$$D \text{ is a virtual image of } D' \text{ and } \frac{1}{D'} - \frac{1}{D} = \frac{1}{F_E}$$

$$\frac{D}{D'} = 1 + \frac{D}{F_E} = \text{magnification } M_E$$

(ii). The observer keeps his eye at rest. This is the more important case since the microscope should be used with accommodation relaxed.

The rays leaving the lens must enter the eye in parallel bundles. Therefore the lens must convert rays leaving D' into a parallel bundle. Therefore D' is at the principal focus of the lens.

$$\therefore \quad D' = F_E \text{ and magnification } = \frac{D}{D'} = \frac{D}{F_E} = M_L$$

If an intermediate degree of accommodation, as may occur in presbyopia, is used the magnification will be intermediate between these values.

(b). **Compound Microscope.**

(i) Objective magnification (M_o). Let i = distance of image from lens; o = distance of object from lens; L = (optical) tube-length; F_o = focal length of objective.

$$\text{Magnification given by a lens } = \frac{i}{o}$$

In the microscope $i \simeq L$ and $o \simeq F_o$

$$\therefore \quad M_o \simeq \frac{L}{F_o}$$

(ii) Eyepiece magnification (M_E)

The eyepiece acts as a simple microscope on the first image

$$M_E = \frac{D}{F_E}$$

$$\text{Total magnification} = M = M_O \times M_E = \frac{L\,D}{F_O\,F_E}$$

From a viewpoint adopted by Abbé, the total magnification may be divided differently between objective and eyepiece. The objective magnification is taken as $\frac{D}{F_O}$ and the eyepiece magnification $\frac{L}{F_E}$. The total magnification is, of course, the same, on either system, namely $\frac{L\,D}{F_O\,F_E}$. Some manufacturers denote their objectives and eyepieces according to this system.

An Alternative Treatment.

The microscope may be taken as equivalent to two lenses of focal length F_O and F_E at a distance L apart. Such a combination has an effective focal length F such that

$$\frac{1}{F} = \frac{1}{F_O} + \frac{1}{F_E} - \frac{L}{F_O\,F_E} = \frac{F_O + F_E - L}{F_O\,F_E}$$

This combination acts on the object as a simple magnifying glass giving magnification $\frac{D}{F}$.

$$\text{Total magnification} = \frac{D}{F_O\,F_E}(F_O + F_E - L) \simeq \frac{D\,L}{F_O\,F_E}$$

Note on Axial Magnification.

If the object moves a distance δo along the axis towards the lens, the image moves back a distance δi. $\frac{\delta i}{\delta o}$ is called the axial magnification.

$$\text{For a real image } \frac{1}{o} + \frac{1}{i} = \frac{1}{f}$$

$$\therefore \frac{1}{o^2}\,\delta o + \frac{1}{i^2}\,\delta i = 0$$

$$\frac{\delta i}{\delta o} = \frac{i^2}{o^2} = M^2$$

The axial magnification is thus equal to the square of the transverse magnification.

(2) Numerical Aperture.

(a) The N.A. of a cone of rays is unchanged when the cone passes through a plane refracting surface unless condition (b) is infringed.

N.A. of pencil (Fig. 23) in glass $= n \sin \alpha$.

" " " " air $= \sin \beta$.

By Snell's law $\frac{\sin \beta}{\sin \alpha} = \frac{\text{air}}{\text{glass}} n$

$\therefore \sin \beta = n \sin \alpha$

$\therefore$ N.A. is unchanged by refraction at a plane surface.

(For the effect of curved refracting surfaces see Footnote p. 81).

(b). The maximum possible N.A. for a cone is n where n refers to the medium through which the cone is travelling. For the N.A. is defined as $n \sin \alpha$ and the sine of an angle is never $> 1{\cdot}0$.

(c). If a cone has N.A. $> 1{\cdot}0$, passage through an air film reduces the N.A. to $1{\cdot}0$. Consider a cone of rays diverging from O (fig. 23). If its N.A. $> 1{\cdot}0$ it must be travelling through a material denser than air. $\therefore \beta > \alpha$.

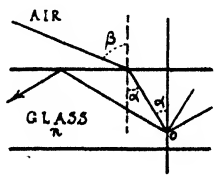


Fig. 23.

If the refracted ray is to emerge, β cannot exceed 90°, For the extreme ray of the cone which emerges, $\sin \beta = 1{\cdot}0$.

By Snell's law $n \sin \alpha = \sin \beta = 1{\cdot}0$.

Thus the N.A. of the part of the cone which emerges is 1·0. The outer rays of the illuminating cone are retained in the glass by total internal reflection at the glass/air surface.

N.A. of Lens.

The N.A. of a lens is the N.A. of the largest cone of light which the lens can take in from an object point at the principal focus.

(d). The N.A. of a lens combination is equal to the radius of the back lens divided by the focal length. (In the case of a simple lens of small N.A. this is obvious from the definition of N.A.)

Let n be the refractive index of the medium on the object side of the lens. Let R be the radius of the (back) lens (see Fig. 24).

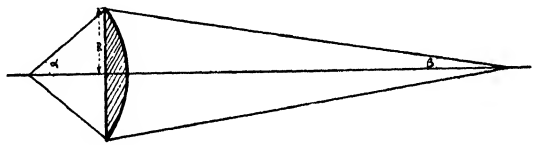


FIG. 24,

By the sine condition (see p. 81) $n \sin \alpha = M_0 \sin \beta$

In an objective $n \sin \alpha = \text{N.A.}$ $M_0 = \dfrac{L}{F_0}$

$$\therefore \text{N.A.} = M_0 \sin \beta \simeq M_0 \frac{R}{L} = \frac{R}{F_0}$$

$$\therefore \text{N.A.} = \frac{\text{radius of back lens}}{\text{focal length}}$$

(e). Brightness of the image. The quantity of light going to form each point on the image is proportional to the solid angle of the cone β, i.e., to $\sin^2 \beta$, since β is always small. (See p. 82).

$$M_0 \sin \beta = n \sin \alpha = \text{N.A.} \quad \therefore \sin^2 \beta = \left(\frac{N.A.}{M_0}\right)^2$$

$$\therefore \text{Brightness of image} \propto \left(\frac{N.A.}{M_0}\right)^2$$

Thus to retain an image of constant brightness, the objective N.A. should increase proportionally to the total magnification.

(3) Depth of Focus.

Two factors require notice—(i.) the accommodation depth; (ii.) depth due to allowable diffusion in the image. The first is essentially subjective; the second alone is operative in photomicrography.

(i.) The final image may theoretically lie anywhere between infinity and the near point.

When the eye is at rest the final image is at a distance F_E from the eyepiece.

When the eye is fully accommodated let it be at a distance o' from the eyepiece. The final image is then at D.

$$\frac{1}{o'} - \frac{1}{D} = \frac{1}{F_E}$$

$$\therefore \quad o' = \frac{D F_E}{F_E + D}$$

$\therefore$ Allowable range for first image $= o' - F_E$

$$= \frac{F_E^2}{F_E + D} \simeq \frac{F_E^2}{D}$$

Corresponding range of thickness in the object

$$= \frac{o' - F_E}{\text{axial magnification}} = \frac{F_E^2}{D M_o^2}$$

$$= \frac{D}{M^2}$$

(ii.) The circle of diffusion corresponding to each point in the object will be inappreciable if smaller than 0.25 mm. diameter in the final image, *i.e.* if $\frac{1}{4M_E}$ mm. in diameter in first image. Suppose this is the case when an image point is at distance δi behind the image plane focussed. The object corresponding to this point is then at distance δo in front of the object plane focussed and $\frac{\delta i}{\delta o}$ = axial magnification $= M_o^2$.

Also, diameter of circle of diffusion $= 2\delta\iota \sin\beta = \frac{1}{4M_E}$ mm.

$$\frac{1}{4M_E} = 2\sin\beta\delta\iota = 2\sin\beta\, M_o^2\,\delta o = 2\,M_o^2\,\delta o \times \frac{n\sin\alpha}{M_o} = 2\,M_o A\,\delta o.$$

$$\therefore \quad \delta o = \frac{1}{8\,M.A.}$$

The aggregate depth of focus is the sum of these terms and is given by $\frac{D}{M^2} + \frac{1}{8\,M.A.}$. The following table shows the depth of focus of several typical lens combinations. If the refractive index of the mounting medium is different from that of the immersion medium, these values should be multiplied by a factor equal to the ratio of these indices to allow for refraction at the cover slip.

Objective	N.A. A	Eyepiece	Total magn. M	Depth of Focus = $\frac{D}{M^2} + \frac{1}{8\,M.A.}$ (m.m.)
$\frac{3}{8}$″ (16 m.m.)	·30	× 8	80	$\frac{1}{26} + \frac{1}{192}$ (m.m.)
$\frac{1}{6}$″ (4 m.m.)	·70	× 8	320	$\frac{1}{400} + \frac{1}{1792}$ (m.m.)
$\frac{1}{12}$″ (2 m.m.)	1·40	× 8	640	$\frac{1}{1600} + \frac{1}{7168}$ (m.m.)

The diffusion depth $\frac{1}{8\,M.A.}$ is thus of small importance compared to the accommodation depth $\frac{D}{M^2}$. Analysis in terms of the wave theory of light shows that the depth of focus is actually somewhat greater than the above simple theory indicates.

(4) Ramsden Circle.

Fig. 6 shows that rays which leave a point on the objective together meet again at a point on the Ramsden disc which is therefore the image of the back lens of the objective formed by the eyepiece. To find its position—

$$\frac{1}{i} = \frac{1}{F_E} - \frac{1}{L} = \frac{L - F_E}{LF_E} \qquad \text{(i).}$$

Let the radius of the back lens be R.

$$\text{Radius of image} = R \times \frac{i}{L} = \frac{RF_E}{L - F_E} \simeq \frac{RF_E}{L}$$

$$\text{Objective N.A.} = \frac{R}{F_O} = A$$

$$\therefore \quad \frac{RF_E}{L} = \frac{AF_O F_E}{L} = A\frac{D}{M_O M_E} = A\frac{D}{M}$$

Hence if M is increased the Ramsden disc is reduced in size. Also by (i.) if F_E is decreased the Ramsden disc moves closer to the eye lens.

Ex. Suppose M is increased to the limit 1000 A.

$$\text{Radius of Ramsden disc} = A\frac{D}{M} = \frac{250}{1000}\ mm. = 0{\cdot}25\ mm.$$

(5) Chromatic Correction.

When two lenses of powers P_1 and P_2 are placed in contact they are equivalent to a lens of power P where

$$P = P_1 + P_2 \qquad \therefore \quad \delta P = \delta P_1 + \delta P_2$$

If the combination is to be achromatic $\frac{\delta P}{\delta \lambda}$ must be zero

$$P_1 = (n_1 - 1)\ K_1 \qquad P_2 = (n_2 - 1)\ K_2$$

(The K's depend on the radii of the curvature of the faces of the lens)

$$\therefore \quad K_1\, \delta n_1 + K_2\, \delta n_2 = O \quad \text{or} \quad P_1 \frac{\delta n_2}{n_2 - 1} + P_2 \frac{\delta n_1}{n_1 - 1} = O$$

gives the condition for achromatism.

Thus achromatism demands $\frac{P_1}{P_2} = \frac{\text{Dispersive power of } (i)}{\text{,, ,, } (ii)}$

From (i)—if $\frac{\delta n}{\delta \lambda}$ is constant, or if, $n_1 = f_1(\lambda)$, $n_2 = f_2(\lambda)$ and $f_1(\lambda) = R\, f_2(\lambda)$, then P is the same for all values of λ.

The condition that $\frac{f_1(\lambda)}{f_2(\lambda)} = R = \text{constant}$, is not satisfied by

any known optical materials and so the spectrum, instead of contracting to a point, is merely shortened to the so-called secondary or tertiary spectrum according to the degree of correction.

(6) The Sine Condition and Aplanatism.

Fig. 25 shows part of the spherical surface separating media of refractive indices n_1 and n_2 its centre of curvature being C. I is the image of O i.e ray OC, which is undeviated, intersects ray OP after refraction at the surface, at I.

For aplanatism it is necessary that all rays from O should pass through the same point I irrespective of the size of α.

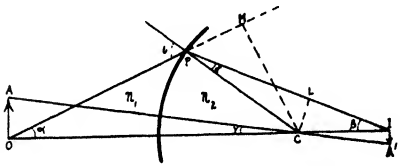


Fig. 25.

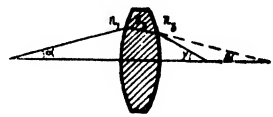


Fig. 26.

For formation of a satisfactory image of an object of finite size it is also necessary that all rays coming from A should be brought together at A'. That is, the magnification must be the same whatever the zone by which a ray from A enters or leaves the lens. It can be shown that this will be the case for a lens only if

$$n_1 \sin \alpha = M n_2 \sin \beta$$

where M is the magnification. This is known as the "Sine Condition" and objectives must be designed so as to satisfy it. In Fig. 26, a ray from the object enters the objective so as to make an angle α with the axis and leaves it making an angle γ. Then

$$n_1 \sin \alpha = M_0 n_3 \sin \gamma = M_0 \sin \gamma$$

since the ray emerges finally into air for which $n = 1.0$.

Note. $n_1 \sin \alpha = N.A.$ of entering cone

$\sin \gamma = N.A.$ of emerging cone

$\therefore$ from sine condition Emergent $N.A. = \dfrac{\text{Incident } N.A.}{\text{Magnification}}$

Thus the cone of rays entering the eyepiece has a far smaller $N.A.$ than that entering the objective. The angle of the former cone never exceeds 2°.

(7) Resolution.

(*A*) Simple Treatment.

(i). Limit of Resolution. (Fig. 27).

Let λ be the wave length in air of the light used.

If P is on the edge of the central diffraction disc which replaces the ideal point-image of O.

$$PL' - PL = \frac{\lambda}{2}$$

$$LI - PL = PI \sin \beta$$

$$PL' - IL' = PI \sin \beta$$

$$\therefore \quad PL' - PL = 2PI \sin \beta$$

$$\therefore \quad PI = \frac{\lambda}{4 \sin \beta}$$

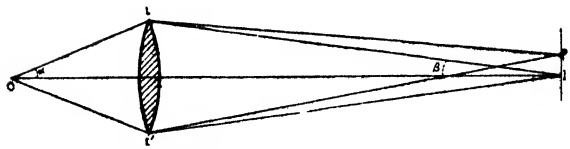


Fig. 27.

For an objective fulfilling the sine condition $n \sin \alpha = M_o \sin \beta$

$$PI = \frac{\lambda}{4 \sin \beta} = \frac{\lambda M_o}{4n \sin \alpha} = \frac{\lambda M_o}{4A}$$

Distance apart of two *object points* whose disc images just touch

$$= \frac{2PI}{M_o} = \text{Limit of Resolution} = \frac{\lambda}{2A}$$

(ii). Limit of useful magnifica.....

This limit is reached when the disc images are visible in the eyepiece

i.e., when $M_E \times 2IP$ = limit of visual acuity = $.25mm. = 250\mu$

or when $$\frac{M_E \times M_o \times \lambda}{2A} = 250\mu$$

$\therefore$ Limit of useful magnification $= M_E \times M_o = \frac{2A}{\lambda} \times 250$

$$= 1000A$$

The value of λ is here taken as $0{\cdot}5\mu$

(*B*) **Abbe Theory.** (Fig. 28).

Light incident at angle θ on a periodic structure of mesh d cm in a medium of refractive index n forms its first diffraction maximum at angle ϕ where

$$nd\ (\sin\theta + \sin\phi) = \lambda$$

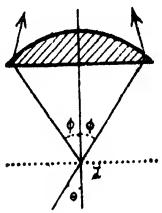


FIG. 28.

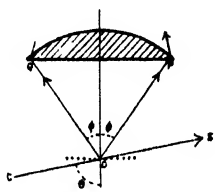


FIG. 29.

Resolution is possible when the transmitted ray and the first diffracted ray both enter the objective, i.e. when

$$d = \frac{\lambda}{n(\sin\theta + \sin\phi)} = \frac{\lambda}{\text{condenser } N.A. + \text{objective } N.A.}$$

This becomes $\frac{\lambda}{2A}$ if the objective is supplied with a full cone of light.

This gives the limit of resolution: the limit of useful magnification follows as in (*A*).

Application to dark-ground illumination. (See Fig. 29).

The undiffracted beam *COS* from the condenser is not received by the objective, and for resolution the first and second diffracted beams *OP* and *OQ* are employed.

$$\text{For first beam } n_1 d\,(\sin\theta - \sin\phi) = \lambda$$

$$\text{,, second ,, } n_1 d\,(\sin\theta + \sin\phi) = 2\lambda$$

$$\therefore 2n_1 d \sin = \phi\lambda = n_1 d\,(\sin\theta - \sin\phi)$$

$$\therefore \sin\theta = 3\sin\phi$$

Hence for the greatest resolution of which the objective is capable, a dark ground condenser should have an *N.A.* three times the objective *N.A.*

INDEX

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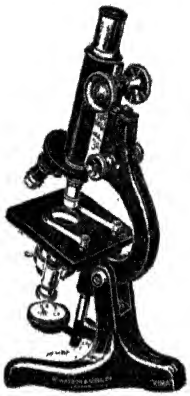

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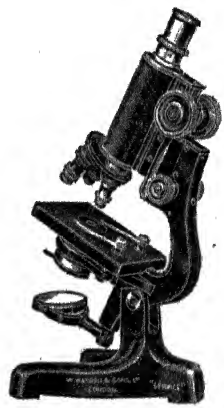

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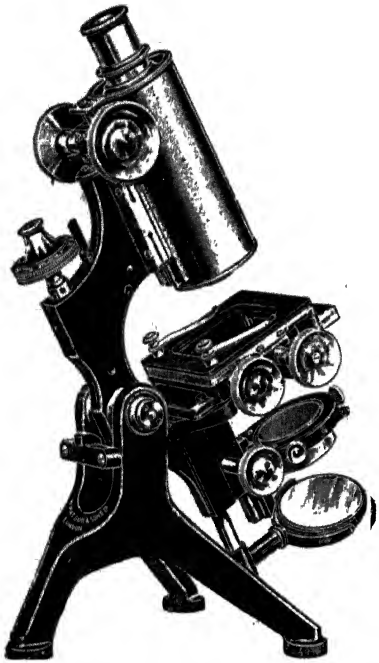

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